GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gscbps.gsconlinepress.com/

(RESEARCH ARTICLE)

Check for updates

# ANTIPLASMODIAL ACTIVITIES OF MORINDA LUCIDA BENTH. (RUBIACEAE) LEAF AND CHARACTERISATION OF ITS LIPOPHILIC CONSTITUENTS

Samuel Akintunde Odediran [1, 2, *], Kayode Oluseun. Akande [1] and Thomas Oyebode Idowu [3]

[1] Department of Pharmacognosy and Natural Products, College of Pharmacy, Afe Babalola University, Ado Ekiti.
[2] Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife.
[3] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife.
* Corresponding Author

ORCID Details
Odediran Samuel: https://orcid.org/0000-0002-8377-7899.
Akande, Kayode: https://orcid.org/0009-0009-3986-5339.
Idowu, Thomas: https//orcid.org/0000-0002-8154-7432

GSC Biological and Pharmaceutical Sciences, 2026, 36(03), 190–199

Publication history: Received on 05 July 2026; revised on 16 August 2026; accepted on 18 August 2026

Article DOI: https://doi.org/10.30574/gscbps.2026.36.3.0287



## ABSTRACT

**Background to the study:** The study investigated the isolation of the lipophilic constituent(s) from the leaf extract of *M. lucida* following the evaluation of its antiplasmodial activities using the three treatment models, with a view to comparing the activities across the models and contributing to the phytochemical knowledge of the plant.

**Methods:** The fresh leaves of *M. lucida* were collected, authenticated, dried, powdered, and macerated in methanol. The antiplasmodial activities were determined in mice infected with *Plasmodium berghei berghei* parasite using the prophylactic, chemosuppressive and curative models. Subsequently, the powdered leaves were macerated in ethyl acetate and concentrated *in vacuo* to a reduced volume (100ml) to which 300ml of methanol was added to obtain a 28.3g precipitate. The precipitate was dissolved in a mixture of $CHCl_3$-MeOH (1:1) and partitioned with n-hexane. The $CHCl_3$-MeOH fraction which contained the lipophilic components was fractionated using standard chromatographic technique.

**Result:** The chromatographic fractions, monitored by vanillin-sulphuric acid spray reagent resulted in the isolation, characterization and identification of phytol and an unidentified ketohydrocarbon using nuclear magnetic resonance. The *M. lucida* extract gave the highest prophylactic, chemosuppressive and curative activities at 200mg/kg, 100mg/kg and 400mg/kg respectively; and the effective doses, $ED_{50}$/$ED_{90}$ elicited respectively imply that an optimum dose of 295mg/kg can be used for any of the models.

**Conclusion:** In conclusion, *M. lucida* leaf extract contains phytol in addition to other lipophilic constituents and elicited significant antiplasmodial activities in all the models.

**Keywords:** *M. Lucida* Leaf Extract, Antiplasmodial Activities, Phytol, Lipophilic Constituents

## 1 INTRODUCTION

Many investigations into plants either for therapeutic actions or for isolation of their constituents have resulted in an array of isolated constituents available for evaluation as therapeutic agents. Most chemical constituents produced by plants have been exploited by humans over the years and continue to be utilized for therapeutic purposes [1]. Investigations are often not exhaustive especially when such are focused on therapeutic agents through bioactivity-guided isolation methods. In other words, plants that have been reported to be thoroughly investigated may still have some hidden phytochemicals locked up in them yet to be tapped. Such constituents may be revealed when research methods and objectives are modified.

*Morinda lucida,* a well-known African tropical plant commonly called Brimstone tree has many medicinal uses from which many compounds have been isolated [2]. Many of the reported secondary metabolites include alkaloids, tannins, anthraquinones, sterols, saponins, polyphenols, terpenoids, phenols and cardiac glycosides[3]. The *in vitro* and *in vivo* experimental studies on various extracts, fractions and isolated compounds of *M. lucid*a support the acclaimed pharmacological activities of the plant, such as antimalarial, antidiabetic, hypotensive, anti-inflammatory, immunostimulatory, antioxidant, antimicrobial, antiproliferative, cognitive-enhancement, anti-sickling, anti-trypanosomal, anti-onchocercid, muscle relaxant, antifungal and anti-leishmanial activities[3]. These evidence-based scientific reports lend credence to their traditional uses [3]. Several studies on the antiplasmodial activities of different morphological parts of *M. lucida* have confirmed the activities in reducing parasite burden [4]. but have not comparatively evaluated the leaf extract across the three antimalarial models to determine the doses at which the plant leaf extract can exhibit prophylactic, chemosuppressive or curative activities. Also, most isolation studies have concentrated on methanol or ethanol extract as starting point and most of the times with non-polar n-hexane extracts frequently implicated as the active fractions [4,5,6].

Apart from the fact that the pharmacological activities of some compounds isolated from the plant are limited, it was also insinuated that some polar constituents may mask the activities of non-polar extracts in this regard. It was on this premise that an attempt was made to isolate lipophilic constituents from the ethyl acetate extract of *M. lucida* (a different approach) [4]. This approach may not only facilitate the identification of new compounds but also enable efforts at future research to be focused on other aspects like the mechanisms of action of these active principles thereby enhancing their potential application in rational drug design [7]. Most of the works done on *M. lucida* either started extraction with non-polar hexane and followed sequentially with solvents of increasing polarity [8,9].

It is possible that additional compounds may be isolated if the extraction methods earlier used are modified or changed entirely. In this work, the method used involved first extraction with moderately polar ethyl acetate, followed by precipitating with a more polar methanol before dissolving the precipitate with a mixture of $CHCI_3$-MeOH (chloroform–methanol 1:1) and partitioning with n-hexane before purification with column chromatography.

## 2 MATERIAL AND METHODS

### 2.1 Plant Collection, Authentication and extraction

The leaves of *Morinda lucida* (L) Benth were collected behind the Faculty of Pharmacy, Obafemi Awolowo University Campus, Ile-Ife. The plant parts were identified by Mr. T. A. Oladele of the Department of Pharmacognosy, Obafemi Awolowo University and authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria with voucher number IFE 5672. The leaves were air-dried for three days, ground into coarse powder, and stored until use. The powdered leaves (0.64kg) were extracted with methanol (3 x 5 L) at room temperature for 72 hours and the combined filtrates were concentrated to dryness under reduced pressure (*in vacuo*) for the antiplasmodial studies. A separate portion of the powdered leaves was extracted with ethyl acetate for phytochemical studies.

### 2.2 Mice

A total of ninety (90) Swiss albino mice weighing between 18 and 24 g (male and non-pregnant female) were purchased from the Animal House, College of Health Sciences, Obafemi Awolowo University, Ile–Ife. (30 animals per experimental model) The animals were housed in aluminum cages under a 12 h light / 12 h dark cycle and fed with standard grower's marsh and provided clean drinking water *ad libitum* and allowed to acclimatize for at least 10 days before commencement of the experiment. The animals were randomly assigned to six groups (I-VI) with five mice in each group for each antiplasmodial experimental model [10].

### 2.3 Parasite

A donor mouse infected with *Plasmodium berghei berghei* NK 65, maintained by serial intraperitoneal passaging in mice, was obtained from the Institute of Advanced Medical Research and Training (IMRAT), University College Hospital, Ibadan. The mouse was euthanized after attaining at least 30 % parasitaemia; the blood obtained was diluted so that 0.2 ml will contain $1 \times 10^7$ infected erythrocytes. Each of the acclimatized and grouped mice for each of the experimental models was inoculated each with 0.2 ml of the diluted blood at appropriate times in the experiment[10].

### 2.4 Preparation of the extracts and standard drug

The extract was weighed in a proportion of 40mg, 80mg, 160mg, 320 mg in 4.0 ml of distilled water; 50mg,100mg, 2000mg, 400 mg in 5.0 ml of distilled water, 60.0 mg, 120mg, 240. 0 mg, 480 mg in 6.0 ml each solubilized with the aid of Tween 80 obtain final doses of 100, 200, 400 and 800 mg kg-1 each respectively for the prophylactic, chemosuppressive and curative tests. In addition, 8.33 mg of chloroquine phosphate was dissolved in small quantity of distilled water and made up to 6.0 mL to give a dose of 10 mg kg-1 for 5 animals to serve as positive control for the curative and the chemosuppressive experiments while pyrimethamine at a dose of 1.2mg/kg was positive control for the prophylactic test[11].

### 2.5 Antimalarial activities of the extract

For each of the models employed, the extract was screened for antimalarial activities at doses 100, 200, 400, and 800 mg kg-1 prepared above. In each case, the solutions were injected to the animals based on the weight.

#### 2.5.1 Prophylactic model

Briefly, on day 1 (D0), Group I mice received 0.2 ml of distilled water while Groups II-V of acclimatised mice received 100, 200, 400 and 800 mg/kg of the extract respectively after recording their rectal temperatures. The procedure above was repeated for two consecutive days (D1-D2). On the fourth day (D3), an inoculum of 0.2 mL of diluted blood containing 1 x $10^7$ *P. berghei* parasitized red blood cells was given to each of thirty (30) test mice intraperitoneally. Seventy-two hours later, mice in Groups I and VI were screened for parasitaemia by taking a smear of blood from the tail [12].

#### 2.5.2 Chemosuppressive model

Briefly, an inoculum of 0.2 mL of diluted blood containing 1 x $10^7$ P. berghei parasitized red blood cells was given to each of thirty (30) test mice intraperitoneally and were randomly divided into six groups (I-VI) (n=5). Two hours later (D0), mice in Groups I and VI were orally administered with distilled water (0.2 mL) and p.o. chloroquine (10 mg/kg) respectively while Groups II-V received 100, 200, 400 and 800 mg/kg of the extract respectively after recording their rectal temperatures using a digital thermometer. The procedure above was repeated for three consecutive days (D1-D3) afterwards[13].

#### 2.5.3 Curative model

Briefly, an inoculum of 0.2 mL of diluted blood containing 1 x $10^7$ *P. berghei* parasitized red blood cells was given to each of thirty (30) test mice intraperitoneally and were randomly divided into six groups (I-VI) (n=5). Seventy-two hours later (D0), mice in Groups I and VI were orally administered with distilled water (0.2 mL) and p.o. chloroquine (10 mg/kg) respectively while Groups II-V received 100, 200, 400 and 800 mg/kg of the extract respectively daily for 5 days, after recording their rectal temperatures. Each day, the parasitaemia for each of the mice were determined[14].

### 2.6 Determination of the level of parasitaemia

For each of the above models, the level of parasitaemia was determined as percentage parasitaemia per dose, after fixing the smear with methanol and staining with Giemsa followed by cell counting using the microscope.

### 2.7 Average percentage parasitaemia and the median effective doses

Each of the Giemsa-stained blood smears was viewed under the microscope using oil immersion (x 100) objective and ten fields with uniform distribution of red cells were counted. For each of the fields selected, the numbers of parasitised (PRBC), as well as unparasitized red blood cells (UPRBC) were counted. The percentage parasitaemia for each field was calculated from the number of parasitised red blood cells divided by the total number of parasitised and unparasitized red blood cells, multiplied by 100. The average value obtained for each mouse and subsequently for each group of the five mice was calculated and recorded as the mean percentage parasitaemia for each group. Percentage reduction in parasitaemia (RDP) or Percentage chemosuppression (CMS) or Percentage clearance (CLR) per dose as the case may be was also determined from the average percentage parasitaemia using the formula: % $chemosupression$ = 100(% parasitaemia $negative\ control$ −% parasitaemia $Test\ dose$ % parasitaemia $negative\ control$ ) The median effective

dose, ED50 and the dose that will cause 90% efficacy, ED90 as measurements of the antimalarial activities of each of the partition fractions/ standard drugs were estimated from the graph of percentage chemosuppression against doses[11,14]. using the Microsoft Office Excel 2010.

### 2.8 Survival times and percentage survivors

The treated mice were observed for mortality for 28 days from the day of drug administration in order to determine the survival times and percentage survivors elicited by the extracts in the mice. The survival time for each mouse was recorded, in days and the average for each group determined as days ± SEM. The percentage survivor or survival rate for each group was estimated from the average survival time as the percentage number of mice eliciting survival time that falls within the average for the whole group[12,14].

### 2.9 Statistical analysis

Values of percentage parasitaemia, percentage chemosuppression and the effective doses for the various extracts were expressed as mean±SEM and analyzed statistically using One-way Analysis of Variance (ANOVA) followed by Student Newman Keul's post-hoc test for multiple comparisons to determine the source of significant differences among the groups. A Value of $p < 0.05$ were considered statistically significant [14].

### 2.10 Extraction, precipitation, and isolation of lipophilic constituents

The powdered plant material (1kg) was macerated in ethyl acetate for 72 h. The extract was then filtered and concentrated *in vacuo* to small volume. Methanol was added slowly to the concentrated extract to precipitate out the crude lipophilic components. The resulting mixture was filtered under vacuum and the precipitate and the supernatant obtained were separately concentrated to dryness and weighed yielding 28.3g and 26.1g respectively to give a total weight of 54.4g. About 19.3g of the precipitate was dissolved in 200 mL of $CHCl_3$ - MeOH (1:1, v/v) and partitioned with (2 × 200 mL) of n-hexane. The resulting hexane fraction (9.8g) and $CHCl_3$ - MeOH fraction (6.7g) after were concentrated to dryness *in vacuo* and weighed. The $CHCl_3$ - MeOH fraction was spotted on TLC plate and developed in $CHCl_3$ - MeOH (1:1 v/v) using vanillin-sulphuric acid as the spray reagent.

### 2.11 Phytochemical analysis of *M. lucida* precipitate

#### 2.11.1 Thin Layer Chromatography (TLC)

The analysis of the extract was performed at ambient temperature using pre-coated silica gel 60 $F_{254}$ plate (Merck). A concentrated solution of the mixture in $CHCl_3$ - MeOH-(1:1, v/v), and the sample was applied at the origin of the TLC plate using a capillary tube while the TLC chamber was allowed to equilibrate with the solvent system. The TLC plate was developed in the selected solvent system inside the closed airtight TLC tank. The TLC plate was then removed, the solvent front was immediately marked and the spots on the plate were visualized under daylight, ultraviolet light (UV) ($\lambda_{max}$254nm) and/or after spraying with Vanillin-Sulphuric acid reagent. The retention factor ($R_f$) was calculated as the ratio of the distance travelled by analyte to the distance travelled by solvent front from the origin.

$$Rf = \frac{\text{Distance travelled by the analyte from the origin}}{\text{Distance travelled by the solvent front from the origin}}$$

#### 2.11.2 Accelerated Gradient Chromatography (AGC)

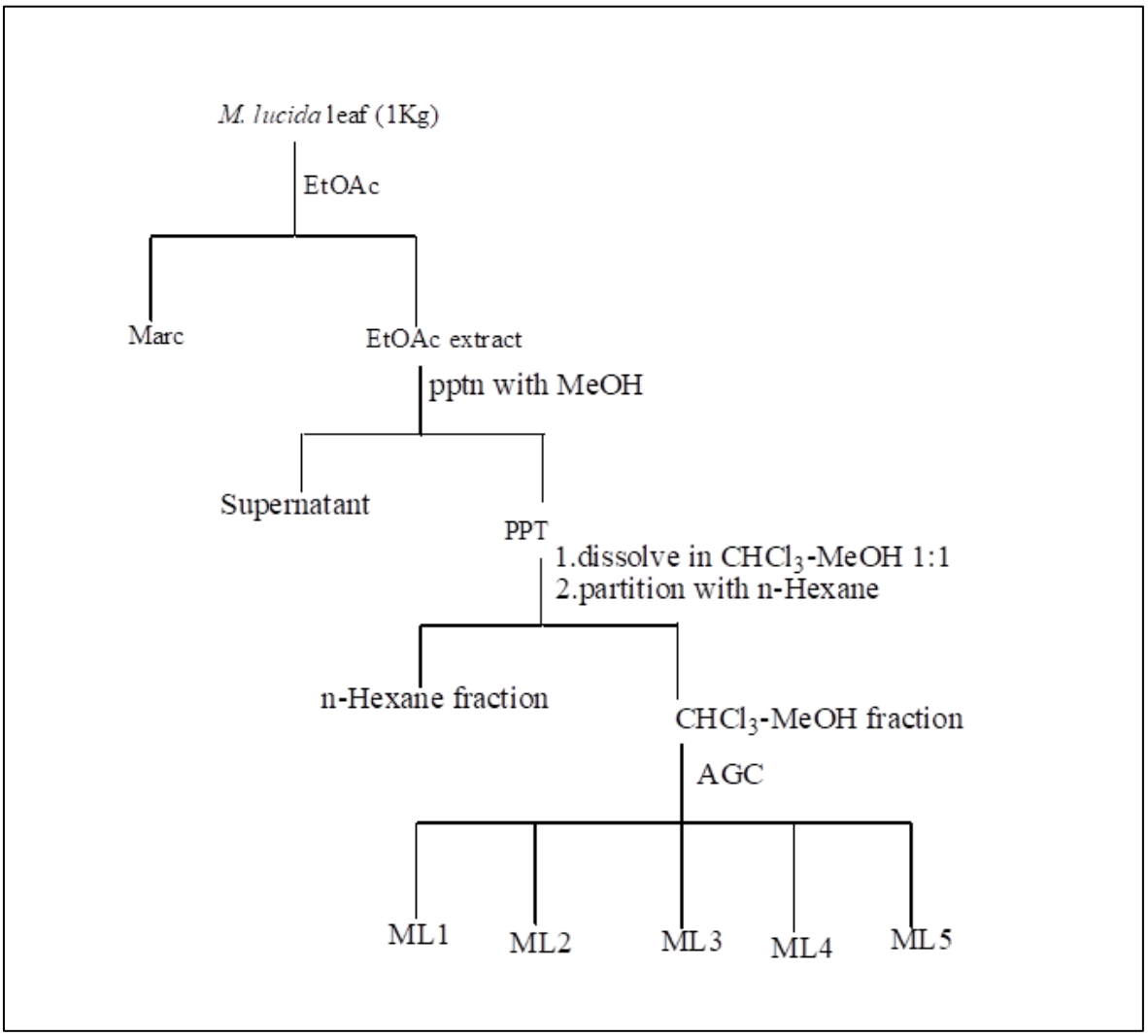
The $CHCl_3$ - MEOH fraction (5.0 g) was dissolved in a chloroform - methanol mixture, pre-adsorbed onto silica gel, and left at ambient temperature to allow complete evaporation of the solvent. The dried, pre-adsorbed sample was subsequently subjected to accelerated gradient chromatography (AGC) using petroleum spirit, dichloromethane and methanol as the eluting solvents. The fractions collected were analysed by TLC using an appropriate solvent system. Fractions exhibiting similar TLC profiles and retention factor (Rf) values were pooled [15].

#### 2.11.3 Column packing

The dried pre-adsorbed sample was transferred into the AGC glass column, and compacted with the aid of the piston, after which a seal was placed on top of the packed sample. Silica gel (Merck, 40-63 microns (µm,), 230-400 mesh) was introduced on top of the packed column and compacted firmly. A seal was positioned to separate the piston from the adsorbent. The piston was then screwed tight to secure the integrity of the packed column. The column was eluted using the appropriate solvent systems [15].

### 2.12 Chromatographic separation

Adsorption column chromatography was performed in ascending mode using the AGC workstation (Baeckstrom Separo AB, Lidindo Sweden). Petroleum ether–dichloromethane mixtures were used as the eluent. The eluates were collected in test tubes and analyzed by TLC. The fractions were pooled based on the TLC profile and the pure isolate analyzed spectroscopically. TLC was carried out to analyze each fraction for their purity and the solvent system found most suitable to fractionate the component of the mixture was n-hex-DCM 1:1. The fractions were bulked based on TLC, concentrated *in vacuo* using rotary evaporator in a flask. Petroleum ether–dichloromethane mixtures were used as the eluent[17].




**Keys: EtOAc**: ethyl acetate; **$CHCl_3$:** Chloroform; **MeOH:** Methanol; **PPT:** Precipitated ethyl acetate extract; **AGC:** Accelerated Gradient Chromatography; **ML1, ML2, ML3;ML4; ML5**: isolated compounds.

**Figure 1: Flow chart of isolation from *M. lucida* leaf**

### 2.13 The fractions were collected as follows:

**1-13**: Pet spirt 100; **14-19:** Pet spirit: $CH_2Cl_2$ 95:5; **20-24:** Pet spirit: $CH_2Cl_2$ 90:10; **25-31:** Pet spirit: $CH_2Cl_2$ 85:15; Pet spirit: $CH_2Cl_2$ 80:20; **32-36:** Pet spirit: $CH_2Cl_2$ 75:25; **37-42:** Pet spirit: $CH_2Cl_2$ 70:30; **43-48:** Pet spirit: $CH_2Cl_2$ 60:40; **49-53:** Pet spirit: $CH_2Cl_2$ 50:50; Pet spirit: $CH_2Cl_2$ 40:60; **54-60:** Pet spirit: $CH_2Cl_2$ 30:70;**61-63:** Pet spirit: $CH_2Cl_2$ 20:80; **63-77:** Pet spirit: $CH_2Cl_2$ 0:100; **MeOH washing:** $CH_2Cl_2$: MeOH 0:100.

### 2.14 Pooling of fractions

Of the above fractions, only those which were observed to be pure on Tlc ($CHCl_3$-MeOH 1:1) were pooled together as follows: **ML1**(1-3, 104 mg; $R_f$ 0.87); **ML2** (6-8, 120 mg; $R_f$ 0.87); **ML3** (17-23, 93 mg; $R_f$ 0.87); **ML4** (31-33, 96 mg; $R_f$ 0.90); **ML5** (55-63, 133 mg; $R_f$ 0.22). On further Tlc analysis with the three solvent systems: n-hex-DCM 1:1, $CHCl_3$-MeOH 1:1, $CHCl_3$-MeOH 7:3, it was observed that ML1 and ML2 were mixtures, ML4 was impure while **ML3** and **ML5** gave one spot each with $R_f$ values of 0.87 and 0.22 respectively.

### 2.15 Spectroscopic analysis of ML3 and ML5

The pure compounds, **ML3** and **ML5** were subjected to spectroscopic analysis of $^{1}$H NMR (Proton Nuclear Magnetic Resonance) at 200MHz and $^{13}$CNMR (Carbon-13 Nuclear Magnetic Resonance) at 50MHz.

## 3 RESULTS

### 3.1 Antiplasmodial Activities

**Table 1: Percentage activity and effective doses elicited by *Morinda lucida* in the different models**

| MODEL | Doses (mg/kg). Mean percentage parasitaemia ± SEM, (Percentage clearance in parenthesis) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.00 | 100.00 | 200.00 | 400.00 | 800.00 | P C | $ED_{50}$ $ED_{90}$ (mg/kg) |
| Prophylactic | 7.72±1.83$^{a}$ (0.00) | 2.51±0.67$^{a}$ (67.49) | 2.76±0.73$^{a}$ (70.34) | 3.39±1.20$^{a}$ (56.09) | 2.97±0.90$^{a}$ (61.53) | 1.92±0.24$^{a}$ (71.53) | 279.35±6.67 452.81±39.95 |
| Chemosuppressive | 44.80±0.49$^{e}$ (0.00) | 7.40±1.90$^{b}$ (83.50) | 20.73±0.60$^{c}$ (51.50) | 17.13±3.66$^{c}$ (61.80) | 27.69±0.79$^{d}$ (38.20) | 3.40±1.70$^{a}$ (92.40) | 294.06±5.12 333.58±53.00 |
| Curative | 9.30±0.05$^{c}$ (0.00) | 2.93±0.24$^{b}$ (68.49) | 2.68±0.26$^{b}$ (71.18) | 2.01±0.16$^{b}$ (78.39) | 2.85±0.39$^{b}$ (69.36) | 1.61±0.28$^{a}$ (82.68) | 294.72±9.30 521.47±32.29 |

**Keys: PC** (positive control: pyrimethamine1.2mg/kg for prophylactic; chloroquine10mg/kg for chemosuppressive and curative; **$ED_{50}$/$ED_{90}$:** Effective doses for each model of test; $^{a, b, c, d}$: same alphabets within the column indicate comparable ($p > 0.05$) activities while different alphabets indicate significant ($p<0.05$) difference in the activities.

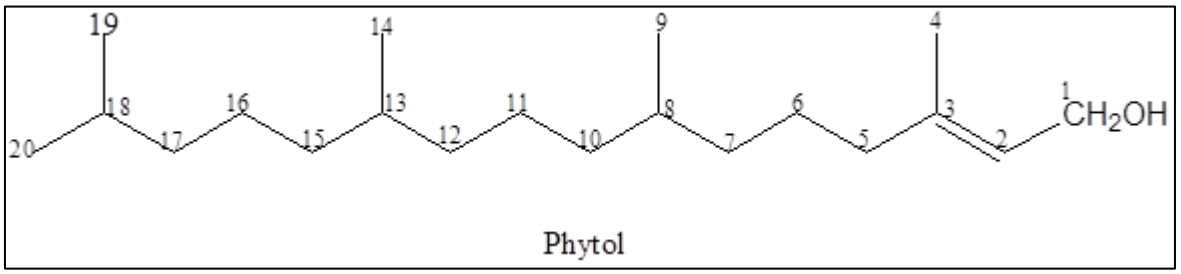
**Table 2: Survival times and percentage survivors (in parenthesis) elicited by *Morinda lucida* in the three models**

| Models/doses | 0.0 | 100.0 | 200.0 | 400.0 | 800.0 |
|---|---|---|---|---|---|
| Prophylactic | 18.4±1.24 (60%) | 18.00±2.52$^{a}$ (67.7%) | 16.33±3.18$^{a}$ (33.3%) | 21.67±1.67$^{a}$ (67.7%) | 24.00±2.31$^{a}$ (50.0%) |
| Chemosuppressive | - | - | - | - | - |
| Curative | 6.5±1.1$^{a}$ (50%) | 17.3±4.1$^{b}$ (50%) | 13.33±0.3$^{b}$ (25%) | 13.0±4.8$^{b}$ (50%) | 10.5±2.1$^{a}$ (50%) |

### 3.2 Spectroscopic data on isolated compounds

**ML5:** **$^{1}$HNMR** (200MHz, $CDCl_3$) in ppm: δ5.39(1H, t,J=6.9Hz,H-2), 4.13(2H,d,J=6.9Hz,H-1), 1.65(3H, s ,H-4), 1.98(2H,t,J=7.6Hz,H-5), 1.49(2H,m,J ,H-6), 0.83(12H,d), 0.95-1.45 (CH; $CH_2$ ,m).

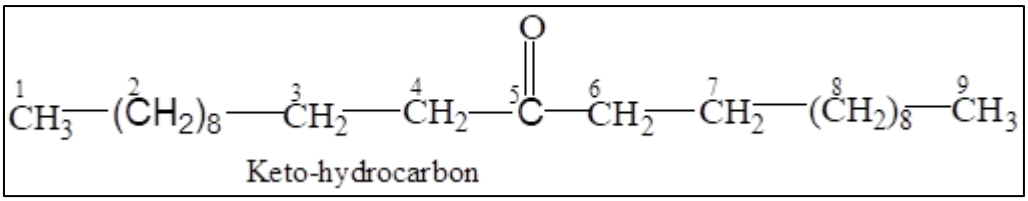
**$^{13}$CNMR** (50MHz, $CDCl_3$) in ppm: δ59.64(C-1), 140.51(C-2), 123.30(C-3), 39.59(C-4), 40.09(C-5), 36.89(C-6), 33.01(C-7), 32.91(C-8), 24.69(C-9), 28.20(C-10), 24.69(C-11), 22.69(C-12), 19.97(C-14), 16.40(C-20)



Phytol

ML3: **$^{1}$HNMR** (200MHz, $CDCl_3$) in ppm: δ2.33 (4H,t,H-4,6),1.63(4H,m,H-3,7), 1.28(16H, H-2,8),0.83(6H,t,H-1,9) [16,17].

**$^{13}$CNMR** (200MHz, $CDCl_3$) in ppm: δ179.7(C-5), 54.0(C-4,6), 34.21(C-3,7), 14.34(C-1,9).




## 4 DISCUSSION

### 4.1 Antiplasmodial activities of M. lucida

The three antimalarial treatment models, namely prophylactic, chemosuppressive and curative models, were employed in this study with the aim of comparing their activities and probably identifying the most appropriate model and the optimum dose for further investigations and potential herbal drug development. The isolation studies may confirm previously reported isolated constituents of the plant or lead to the identification of additional bioactive compounds. The prophylactic activity studies showed that the extract, exhibited parasitaemia level which is comparable to that of pyrimethamine (1.2 mg/kg), the positive control at all the doses tested. The percentage reduction in parasitaemia, however showed that only at 200 mg $kg^{-1}$, was the activity comparable to that of the positive control. The other doses tested also elicited appreciable reductions in parasitaemia Table 1. These findings suggests that the leaf extract of *M. lucida* possesses considerable prophylactic potential against malarial infection. In the chemosuppressive experiment, the *M. lucida* leaf extract at 100 mg/kg, the lowest dose tested, produced a chemosuppression of 83.5 % compared to 92.4% elicited by chloroquine (10.0 mg/kg), positive control drug alluding to the high chemosuppressive potential of the extract. The other doses tested also elicited varying degrees of suppression in parasitaemia, Table 1. In the curative experiment and at all the doses tested, *M. lucida* leaf extract elicited comparable percentage parasitaemia higher than that of the positive control. However, it also gave the highest percentage clearance (78.4 %) at 400 mg/kg [18], obtained optimal antiplasmodial activity 83% at 400 mg/kg); with the other doses comparable to it and chloroquine (10.0 mg/kg), positive control drug, eliciting 82.7% activity Table 1. These results confirm the potent antiplasmodial activity of the extract and suggest that optimum activities were achieved at doses of 200, 100, and 400 mg $kg^{-1}$ in the prophylactic, chemosuppressive and curative drugs respectively.

The corresponding $ED_{50}$ and $ED_{90}$ values for the prophylactic, chemosuppressive, and curative models were 279.35±6.67, 452.81±39.95; 294.06±5.12, 333.58±53.0; 294.72±9.30, 521.47±32mg/kg respectively Table 1. These findings suggest that doses slightly below 300mg/ kg, may produce appreciable antiplasmodial activity across the different treatment models. The relatively prolonged survival times observed in the prophylactic and curative models at 400mg/kg and 100 mg/kg respectively, together with the high percentage survival recorded at 100mg/kg and the favourable median effective doses Table 2 further support the potential of *Morinda lucida* leaf methanol extracts as an effective antimalarial agent across the different treatment models [19].

### 4.2 Isolation of ML5and ML3 from CHCl3 -MeOH fraction of M. lucida

Although *M. lucida* has been reported to contain anthraquinones[19], ursolic and oleanolic acids[20], as well as numerous polar constituents[21], relatively few compounds have been isolated and characterised from its lipophilic fractions. The present study therefore aimed to isolate compounds from the lipophilic fraction of *M. lucida* leaves by exploiting differences in the polarity, affinity and solubility of their constituents' compounds. The extraction procedure employed was a relatively rapid method involving the precipitation of lipophilic constituents from the ethyl acetate leaf extract through the addition of a highly polar solvent like methanol. Highly non-polar constituents are generally expected to exhibit limited solubility in methanol and, therefore, precipitate from the extract under these conditions.

### 4.3 Characterisation and identification of isolates

**ML5:** ML5 is a yellow oil, The $^{1}$HNMR (200 MHz, $CDCl_3$) revealed the presence of an olefinic proton at δH 5.39 (1H, t, J= 6.9Hz, H-2) (corresponding to C-2) coupled to a deshielded neighbouring methylene proton at δ 4.13 (2H, d, J=6.9Hz) (corresponding to C-1). Four methyl groups resonated at δH 0.83 (12H, d) assignable to C-9, C-14, C-19 and C-20 while one methyl group was deshielded downfield at δ 1.65 (3H, s) corresponding to C-4, a deshielded methylene group at δ 1.98 (2H, t, J=7.6Hz) corresponding to C-5, another methylene group at δ 1.49 (2H, m,) corresponding to C-6 and a number of methylene multiplet at δ 0.95-1.45 account for the rest of the methylene group.

The $^{13}$CNMR (200MHz, $CDCl_3$) of ML5 displayed 20 carbon signals which include a highly deshielded olefinic carbon atom at δC 140.5 (C-2), an olefinic quaternary carbon at δ 123.3 (C-3), a deshielded methylene group at δ 59.6 (C-1), another deshielded methylene group at δ 40.1 (C-5) and a deshielded methyl group at δ 39.59 (C-4). Out of the twenty (20) carbon signals observed, the $^{13}$C APT spectrum showed nine (9) signals for $CH/CH_3$, ten (10) $CH_2$ signals, and one (1) quaternary carbon (Cq). The identity of ML5 as phytol was confirmed by comparison of its physical properties and

spectral data ($^{1}$H-NMR and $^{13}$C-NMR) with those reported in the literature[17]. Phytol, (3,7,11,15-tetramethylhexadec-2-en-1-ol), also known as 6,7,10,11,14,15-hexahydrogeranylgeraniol constitutes the lipophilic side chain of chlorophyll and is one of the most prominent member of the linear (acyclic) diterpenes[21]. It serves as a biosynthetic precursor in the formation of chlorophyll and phylloquinone (Vitamin K) [22]. Phytol is a colourless to pale yellow liquid with the molecular formula $C_{20}H_{40}O$ and molecular weight of 296.54 g/mol. It is also used as an ingredient of fragrances used in soaps, cosmetic formulations and other household products. Phytanic acid, a metabolite derived from phytol, accumulates in individuals with Refsum's disease, a rare genetic defect of phytanic acid metabolism[23]. Phytol has been reported to exhibit tumour-promoting activity in ICR mouse skin under specific experimental conditions [24]. However, the presence of phytol in the leaf extract alone does not necessarily imply that the crude extract possesses tumour promoting activity as the overall biological effect of a plant extract depends on the nature, concentration, and interactions of its multiple constituents.

**ML3:** The $^{1}$HNMR (200 MHz, $CDCl_3$) of ML3, revealed the presence of deshielded methylene protons at δH 2.33 (4H, t) assignable to C-4 and C-6 coupled to a less deshielded methylene protons at δH 1.63 (4H, m) corresponding to C-3 and C-7. A broad signal attributable to the methylene protons of a long-chain hydrocarbon (16 $CH_2$) was observed at δH 1.28 and assigned to the intervening methylene carbons between C-2 and C-8. Two terminal methyl groups resonated at δH 0.83 (6H, t), corresponding to H-1 and H-9, and were coupled to the adjacent methylene groups.

The $^{13}$CNMR (200 MHz, $CDCl_3$) spectrum of ML3 displayed signals at δC 179.7, δC 14.3, δC 54 while the remaining carbon resonances appeared within the region δC 22.0 -35.0. These $^{13}$C signals were attributed to a quaternary carbon bearing keto functional group at δC 179.7 corresponding to C-5, two terminal methyl carbons adjacent $CH_2$ to the carbonyl group (C-4, and C-6), and the remaining aliphatic methylene respectively[16]. From the available spectral data ($^{1}$H-NMR and $^{13}$C-NMR), ML3 was tentatively identified as a ketone-containing hydrocarbon. However, additional spectroscopic data like $^{1}$H-$^{1}$H COSY, HETCOR, HMBC, IR and MS are needed to fully characterise the ketone- containing hydrocarbon. Although phytol, is a well-known natural product in phytochemical studies, its occurrence in *M. lucida* leaf has only recently been reported[25].

## 5 CONCLUSION

This study involved the antiplasmodial studies on *M. lucida* leaf across the three treatment models and phytochemical investigation in order to isolate and characterise its lipophilic constituents. Out of the five compounds isolated in this work only two compounds namely phytol and a keto- containing hydrocarbon, were characterized while a dose of 300mg/kg could give good effects across the three treatment models.

## STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

### *Acknowledgments*

The authors acknowledge the Institute of Advance Medical Research and training, University College Hospital, Ibadan for supplying the *Plasmodium berghei berghei* parasite, The Forestry Research Institute of Nigeria for authenticating the plant, the Multidisciplinary laboratory, College of Health Sciences, OAU, Ife for allowing the use of the animal house for the antiplasmodial experiment and the Central Science Laboratory, OAU, Ife for the spectroscopy.

### *Funding Source*

The authors received no financial support for the research, authorship, and/or publication of this article.

### *Disclosure of conflict of interest*

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### *Statement of ethical approval*

All procedures involving animals in this study were conducted in accordance to the Health Research and ethics Committee (HREC), Institute of Health, Obafemi Awolowo University, Ile Ife Nigeria protocol number HREC No. IPHOAU/12/404 of April 7, 2015.

### *Statement of informed consent*

There are no human subjects in this article and informed consent is not applicable.

### *Author Contributions*

- **Odediran Samuel:** Conceptualization, Methodology, Visualization, Investigation, Formal analysis, Data curation, Software, Writing–original draft, Validation
- **Akande, Kayode :** Methodology, Investigation, Data curation, Validation.
- **Idowu, Thomas:** Conceptualization, Methodology, Formal analysis, Data curation, Software, Validation.

### *Statement of Human and Animal Rights*

This article does not contain any studies with human subjects. All procedures in this study were conducted in accordance to the Health Research and ethics Committee (HREC), Institute of Health, Obafemi Awolowo University, Ile Ife Nigeria.

## REFERENCES

[1] Yangzom S, Sharma M, Sharma M, Sharma M. Therapeutic uses and pharmacological properties of medicinal plants and their natural products. Springer Nature Link, Singapore 2026; 763-806, https://doi.org/10.1007/978-981-97-9859-9_38.

[2] Adeleye OO, Ayeni OJ, Ajamu MA. Traditional and Medicinal uses of *Morinda lucida*. Journal of medicinal plants studies 2018; 6(2) : 249-254.

[3] Adewole KE, Attah AF, Adebayo JO . *Morinda lucida* Benth (Rubiaceae): A review of its ethnomedicine, phytochemistry and pharmacology. Elsevier Journal of ethnopharmacology 2021; 276:114055. doi:10.1016/j.jep.2021.114055. Epub 2021 Mar 19.

[4] Oladeji OS, Oluyori AP, Dada AO. Antiplasmodial activity of *Morinda lucida* Benth. leaf and bark extracts against *Plasmodium berghei* infected mice. Saudi Journal of biological science 2022 ; 29(4) :2475-2482. doi : 10.1016/j.sjbs.2021.12.017. Epub 2021 Dec 13. PMID: 35531230; PMCID: PMC9073002.

[5] Deharo, E,kA Ginsburg, H . Analysis of additivity and synergism in the anti-plasmodial effect of purified compounds from plant extracts. Malaria journal 2011; 10 (1), S5. https://doi.org/10.1186/1475-2875-10-S1-S5.

[6] Ezenyi IC, Verma V, Singh S, Okhale SE, Adzu B. Ethnopharmacology-aided antiplasmodial evaluation of six selected plants used for malaria treatment in Nigeria. Journal of ethnopharmacology 2020; 254:112694. https://doi.org/10.1016/j.jep.2020.112694.

[7] Cirinciani M, Da Pozzo E, Trincavelli ML, Milazzo P, Martini C. Review Drug Mechanism : A bioinformatic update. Biochemical pharmacology 2024; 228: 116078. doi: 10.1016/j.bcp.2024.116078.

[8] Nwokonkwo DC, Nwafor EI. Extraction, isolation and characterization of secondary metabolites in the leaves of *Morinda lucida* from Oshiegbe in Ebonyi State. Physical sciences reviews 2022; 8 (11):226, 13pp. doi : 10.1515/psr-2021-0226

[9] Iyekowa IAOI, Ruth OO, Oghomwen TM, Lilian E, Amoren IFA, Lucky OE, Agholor MO, Ferdinand OA, Justina OA, Ndubuisi P E, Ogbodhu CI. GC-MS Analysis and Antifungal Activity of *Morinda lucida* Extract. NIPES - Journal of science and technology research 2024; *6*(3), https://doi.org/10.5281/zenodo.14009879.

[10] Uraku AJ, Hepatoprotective Effects of *Plasmodium berghei* Infected Swiss Mice Treated with some Plant Extracts. Journal of pharmacy and allied health sciences 2016; 6:1-7, DOI: 10.3923/jpahs.2016.1.7

[11] Girmaw F, Engidawork E. *In Vivo* Anti-Malarial Activity of the Aqueous Root Extract of *Euclea divinorum* Hiern (Ebenaceae) against *Plasmodium berghei* ANKA. Evidence based complementary alternative medicine 2022;2640648, doi: 10.1155/2022/2640648.

[12] Orumwensodia KO, Uadia PO. *In vivo* Antimalarial Activity of *Morinda lucida* Benth. Stem bark in *Plasmodium berghei*-infected Mice. Nigerian journal of biochemistry and molecular biology 2022; 37(3), 194-200.

[13] Craig AG, Grau GE, Janse C,Kazura JW, Milner D, Barnwell JW,Turner G, Langhorne J (February2012). "The Role of Animal Models for Research on Severe Malaria. PLOS pathogens 2012;8 (2): e1002401 doi:10.1371/journal.ppat.1002401(https://doi.org/10.1371).

[14] Odediran S.A, Elujoba, A.A. and Adebajo C.A. Influence of formulation ratio of the plant components on the antimalarial properties of MAMA Decoction. Parasitology research 2014; 113:1977-1984.

[15] Jandera, P., Churáček, J., Simple (Continuous) Gradient Elution Chromatography Chapter 4, Journal of chromatography library1985; 31: 67-129.ISSN 0301- 4770, ISBN 9780444421241,https://doi.org/10.1016/S0301-4770(08)60240-7.

[16] Goodman AR, Eric O, Adam A. Assignment in the natural-abundance carbon -13 nuclear magnetic resonance spectrum of chlorophyl a and a study of segmental motion in neat phytol. Journal of the american chemical society, 1973; 95(23):7553-7558. https://doi.org/10.1021/ja00804a003.

[17] Faleye FJ. Phytochemical studies of the extracts of *Citrus paradise* MACFAD seeds and the aerial parts of *Aspilia africana* (PERS) CD ADAMS: Ph.D Thesis 2008, University of Ife, Nigeria.

[18] Afolabi, O.J., Abejide, A.E. Antiplasmodial activities of *Morinda lucida* (Benth) and *Alstonia boonei* (De wild) in mice infected with *Plasmodium berghei*. Bulletin of the national research centre*. 2020;* **44**:85. https://doi.org/10.1186/s42269-020-00342-8.

[19] Adewunmi CO, Adesogan EK. Anthraquinone and oruwacin from *Morinda lucida* as possible agents in fasciolasis and schistosomiasis control. Fitoterapia 1984; 55: 259-63.

[20] Cimanga RK, Tona GL, Mesia GK, Kambu OK, Bakana DP, Kalenda PDT, Penge AO, Muyembe JT, Totte J, Pieters L, Vlietinck AJ. Bioassay- Guided Isolation of Antimalarial Triterpenoid Acids from the leaves of *Morinda lucida*. Pharmaceutical Biology 2006; 44(9):677-681. doi : 10.1080/13880200601009123

[21] Torssell GBK. Natural Product Chemistry, a mechanistic, biosynthetic and ecological approach second edition, Apotekarssocieteten- Swedish Pharmaceutical Society, Sweden Pharmaceutical Press, Sweden, 1997, pp 251-302.

[22] Stroev EA. Biochemistry, revised from the 1986 Russian edition, MIR Publishers Moscow 1989, p90.

[23] Voet D, Voet JG. Biochemistry, Second edition, New York, USA, John Wiley and Sons Inc., 1995, p678.

[24] Masayori K, Chihiro M, Masaaki M. Phytol is a novel tumour promoter on ICR mouse skin. Japanese Journal of cancer research 1999; 90(4):377-384. doi: 10.1111/j.1349.7006.1999.tb00758.

[25] Okwute SK, Ochi IO. Identification of Phytol and Cis-1,4-Polyisoprene from the leaf extracts of Morinda lucida (Rubiaceae). International Journal of pharmacy and chemistry 2025;11(5):94-107. doi:10.11648/j.ijpc.20251105.1.