



## **Phytochemical Screening and Evaluation of Antioxidant and Proximate Properties of *Morinda lucida* Ethanolic Extract**

**Oludare Temitope Osuntokun<sup>1\*</sup>, A. M. Yusuf-Babatunde<sup>2</sup>, O. O. Ige<sup>2</sup>  
and A. E. Odufuwa<sup>3</sup>**

<sup>1</sup>Department of Microbiology, Faculty of Science, Adekunle Ajasin University, Akungba Akoko,  
P.M.B 001, Ondo State, Nigeria.

<sup>2</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife,  
Osun State, Nigeria.

<sup>3</sup>Department of Microbiology, Faculty of Science, Obafemi Awolowo University, Ile Ife, Osun State,  
Nigeria.

### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author OTO is the leading author, who designed, analyzed, interpreted and prepared the manuscript for publication. Author OTO is a researcher who researched the antimicrobial and phytochemical properties of various medicinal plants in Nigerian and Africa. Authors AEO did the proof reading of the entire manuscript to prepare it for publication and also helped during the antimicrobial assays. Authors AMYB and OOI helped during the phytochemical procedure and the chemical analysis of the plant extracts. All authors read and approved the final manuscript.*

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## ABSTRACT

*Morinda lucida* is a tropical West African evergreen shrub or small to medium sized rainforest tree, having brown colored bark, white flowers and yellowish ellipsoid seeds. It is widely used in traditional medicine systems of different countries. This study was designed to carry out the preliminary phytochemical screening and evaluation antioxidant and proximate properties of ethanol extracts prepared from the bark, leaves and root of *M. lucida*. It revealed the presence of saponin, tannins, alkaloids, flavonoids, and cardiac glycosides. Furthermore, it also revealed the presence of minerals like sodium, potassium, calcium, iron, zinc, copper and phosphorus were confirmed. Anti-nutrients were also present in appreciable quantities. The anti-nutrients: Phytates, oxalates and saponins were present in different concentrations, Furthermore, the percentage of proximate content and *In-vitro* antioxidant potential were also estimated in this current study. The study analyzed the elemental composition, anti-nutrient and antioxidant properties of ethanol extracts of the stem, leaf and root of *M. lucida*.

**Keywords:** *Morinda lucida* benth; quantitative methods of analysis; mineral composition; anti-nutrients; proximate method; phytochemical screening; antioxidant properties.

## 1. INTRODUCTION

*Morinda lucida* (Rubiaceae) is an evergreen shrub or small to medium sized rainforest tree also called Brimstone tree. In Cote D'Ivoire, it is locally called Sangogoor Bondoukoualongua while in Ghana, it is known as Twi, Konkroma or Ewe amake. Among the Togolese, Ewe amaka or Atakake, South-Western Nigeria and among the Yoruba, it is called Oruwo [1]. It can grow from 2.4 to 18 metres tall. It has a rough bark, grey in colour, flaking off in irregular patches. Its leaves are about 7 to 15 cm long by 3.5 to 7.5 cm broad, and flowers are white with a narrow glabrous corolla-tube about 2.5 cm.

The stem bark, roots and leaves infusion of *M. lucida* is used as an antimalarial, antidiabetic, jaundice and dysentery treatment [2] and it is used in antimalarial activity [3-6], anti-*Salmonella typhi* activity [7], effect on contractility of isolated uterine smooth muscle of pregnant and non-pregnant mice [8], toxicity and mutagenic studies [9-11] and it has anti-diabetic property [12]. *M. lucida* extracts have been reported to have antioxidant and reducing activities [13], and antimicrobial activity [14,15].

The major constituents of *M. lucida* extracts were found to be: essential oils, anthraquinones and anthraquinols [12]. Oruwalol, oruwal, ursolic acid, and oleanolic acid were also isolated from this plant [3,12,16]. The red colorants of *M. lucida* were confirmed to be 1-methyl-ether-alizarin, rubiadin and derivatives, lucidin, soranjidiol, damnacanthal, nordamnacanthal, morindin, munjistin and purpuroxanthin, anthraquinones, oruwacin, tannins, flavonoids, digitolutein, and

saponosides were isolated from different parts of *M. lucida*. The root of *M. lucida* is used as chewing sticks are for oral hygiene in Nigeria. The aqueous extracts of different parts of this plant were found to be effective against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* [17,18].

Keeping in view the popular use of different parts of *M. lucida* and evidence based confirmation of different folklore medicinal claims, further investigation was considered mandatory. In the present study antioxidant, phytochemical and proximate properties of ethanol extracts *M. lucida* were evaluated and the results are presented in the current communication.

## 2. MATERIALS AND METHOD

### 2.1 Collection and Identification of Plant Sample

The root, leaf and stem bark from *M. lucida* plants were collected from the tropical rain forest Osogbo, Osun State Nigeria (7.7827° N, 4.5418° E). The plants specimen were identified and authenticated at Department of Plant Science and Biotechnology, Adekunle Ajasin University Akungba-Akoko and Obafemi Awolowo University, Ile-Ife, Nigeria. Voucher number AAU-2418 was recorded for the plant extract for future reference.

### 2.2 Preparation of Extract

Fresh leaves, barks and roots of *M. lucida* were collected separately and then air-dried. It was

then hammer milled with Jacobson Ajacs 17 3600 RPM Hammer-mill model 17 DF8. The hammer milled dried samples, 1 kg each was soaked in 5 L each of absolute ethanol for 72 hours and was filtered. The filtrate was concentrated using a rotary evaporator and was stored until required for use.

## **2.3 Qualitative Method of analysis**

### **2.3.1 Preliminary test / preparation test**

The filtrate was used for the phytochemical screening for flavonoids, tannins, saponins, alkaloids, reducing sugars, anthraquinones and anthocyanosides [19].

#### *2.3.1.1 Test for alkaloids*

About 0.2 gram was warmed with 2% of H<sub>2</sub>SO<sub>4</sub> for two minutes, it was filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicated the presence of Alkaloids.

#### *2.3.1.2 Test for tannins*

One milliliter of the filtrate was mixed with 2 mL of FeCl<sub>3</sub>, A dark green colour indicated a positive test for the tannins.

#### *2.3.1.3 Test for saponins*

One milliliter of the filtrate was diluted with 2 mL of distilled water; the mixture were vigorously shaken and left to stand for 10 min during which time, the development of foam on the surface of the mixture lasting for more than 10 mins, indicated the presence of saponins.

#### *2.3.1.4 Test for anthraquinones*

One milliliter of the plant filtrate was shaken with 10 mL of benzene; the mixture was filtered and 5 mL of 10% (v/v) ammonia were added, then shaken and observed. A pinkish solution indicated the presence of anthraquinones.

#### *2.3.1.5 Test for anthocyanosides*

One milliliter of the plant filtrate was mixed with 5 mL of dilute HCl; a pale pink colour indicated the presence of anthocyanides.

#### *2.3.1.6 Test for flavonoids*

One milliliter of plant filtrate was mixed with 2 mL of 10% lead acetate; a brownish precipitate indicated a positive test for the phenolic flavonoids. While for flavonoids, 1 mL of the plant filtrate was mixed with 2 mL of dilute NaOH. A

golden yellow colour indicated the presence of flavonoids.

#### *2.3.1.7 Test for reducing sugars*

One milliliter of the plant filtrate was mixed with Fehling A and Fehling B separately; a brown colour with Fehling B and a green colour with Fehling A indicate the presence of reducing sugars.

#### *2.3.1.8 Test for cyanogenic glucosides*

This was carried out subjecting 0.5 g of the extract 10 mL sterile water filtering and adding sodium picrate to the filtrate and heated to boil.

#### *2.3.1.9 Test for cardiac glucosides*

Legal test and the killer-kiliani was adopted, 0.5 g of the extract were added to 2 mL of acetic anhydride plus H<sub>2</sub>SO<sub>4</sub>.

### **2.3.2 Quantitative method of analysis**

#### *2.3.2.1 Saponins*

About 20 grams each of dried plant samples was ground and, put into a conical flask after which 100 mL of 20% aqueous ethanol was added. The mixture was heated using a hot water bath. At about 55°C, for 4 hour with continuous stirring, after which the mixture was filtered and the residue re-extracted with a further 200 mL of 20% ethanol. The combined extract was reduced to 40 ml over a water bath at about 90°C. The concentrate was transferred into a 250 mL separating funnel and 20 mL of diethyl ether were added and then shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated three times. 60 mL of n-butanol were added. The combined n-butanol extract was washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage of the starting material [20].

#### *2.3.2.2 Flavonoids*

About 10 g of the sample was extracted repeatedly with 100 mL of 80% aqueous ethanol, at room temperature. The whole solution was filtered through What man filter paper No 42. The filtrate were later transferred into a crucible and evaporated into dryness over a water bath; the dry content was weighed to a constant weight [21].

### 2.3.2.3 Cardiac glycosides

Legal test and the killer-kiliani test was adopted. 0.5 g of the extract was added to 2 mL of acetic anhydride plus H<sub>2</sub>SO<sub>4</sub> [20].

### 2.3.2.4 Tannins

About 500 mg of the sample was weighed into a 50 mL plastic bottle and 50 mL of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the marked level. Then, 5 mL of the filtrate was transferred into a test tube and mixed with 2 mL of 0.1M FeCl<sub>3</sub> in 0.1M HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes. The tannins content was calculated using a standard curve technique [22].

### 2.3.2.5 Alkaloids

Five grams of the sample was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was then be added, the reaction mixture was covered and allowed to stand for 4 hour. This was filtered and the extract will be concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation is complete. The whole solution was allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue being the alkaloid, which was dried and weighed to a constant mass [22].

### 2.3.2.6 Phlobatannins

About 0.5 grams of the extract was dissolved in distilled water and filtered. The filtrate was boiled in 2% HCl, red precipitate show the presence of phlobatannins [20].

## **2.3.3 Determination of proximate analysis of medicinal plants**

The proximate parameters (moisture, dry matter, ash, crude fats, proteins and fibers, nitrogen, carbohydrates and energy values) were determined using Association of Official Analytical Chemists (AOAC) methods.

### 2.3.3.1 Determination of moisture content

Determination of moisture content was done by drying samples in oven (Wise Ven, WON-50,

Korea) at 110°C until constant weight was attained [23].

### 2.3.3.2 Nitrogen estimation

Nitrogen estimation was carried out by the micro-Kjeldahl (BUCHI, KjelFlex K-360, Switzerland) method with some modification [24].

### 2.3.3.3 The crude protein

The crude protein was subsequently calculated by multiplying the nitrogen content by a factor of 6.25 [24]. The energy value estimation was done by summing the multiplied values for crude protein.

### 2.3.3.4 Crude fat and carbohydrate

Crude fat and carbohydrate respectively at Water Factors (4, 9 and 4). Crude fat was determined by Soxhlet apparatus using *n*-hexane as a solvent.

### 2.3.3.5 The ash value

The ash value was obtained by heating samples at 550°C in a muffle furnace (Wise Therm, FHP-03, Korea) for 3 h [24].

### 2.3.3.6 The carbohydrate content

The carbohydrate content was determined by subtracting the total crude protein, crude fiber, ash content and crude fat from the total dry matter [23].

### 2.3.3.7 Crude fiber was estimated

Crude fiber was estimated by acid-base digestion with 1.25% H<sub>2</sub>SO<sub>4</sub> (v/v) and 1.25% NaOH (w/v) solutions [25].

## **2.4 Antioxidant Assay**

### **2.4.1 Determination of total phenol**

The total phenol content of the extract determine by the method described by [26]. 0.2 ml of the extract was mixed with 2.5 ml of 10% Folin ciocalteau's reagent and 2 mL of 7.5% Sodium carbonate. The reaction mixture was subsequently incubated at 45°C for 40 mins, and the absorbance was measured at 700 nm with LAMBDA 950 UV/Vis spectrophotometer. Gallic acid was used as standard [25].

#### **2.4.2 Determination of total flavonoid**

The total flavonoid content of the extract was determined using a colourimeter assay. Exactly 0.2 mL of the extract was added to 0.3 mL of 5% NaNO<sub>3</sub> at zero time. After 5 min, 0.6 ml of 10% AlCl<sub>3</sub> was added and after 6 min, 2 mL of 1 M NaOH was added to the mixture followed by the addition of 2.1 mL of distilled water. Absorbance was read at 510 nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent [10].

#### **2.4.3 Determination of ferric reducing property**

The reducing property of the extract was determined by [27]. 0.25 mL of the extract was mixed with 0.25 ml of 200 mM of Sodium phosphate buffer pH 6.6 and 0.25 mL of 1% KFC. The mixture was incubated at 50°C for 20 min, thereafter 0.25 ml of 10% TCA was also added and centrifuge at 2000 rpm for 10 min, 1 mL of the supernatant was mixed with 1 mL of distilled water and 0.1% of FeCl<sub>3</sub> and the absorbance was measure at 700 nm.

#### **2.4.4 Determination of free radical scavenging ability**

The free radical scavenging ability of the extract against DPPH (1, 1- diphenyl-2-picrylhydrazyl) was determined using the method described by [17]. 1 mL of the extract was mixed with 1 mL of the 0.4 mM methanolic solution of the DPPH the mixture was left in the dark for 30 min before measuring the absorbance at 516 nm.

#### **2.4.5 ABTS scavenging ability**

2,2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) (ABTS) scavenging ability. The ABTS scavenging ability of the extract was determined according to the method described by [28,29]. The ABTS was generated by reacting an (7mM) ABTS aqueous solution with K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (2.45 mM/L, final conc.) in the dark for 16hours and adjusting the absorbance at 734 nm to 0.700 with ethanol 0.2 of the appropriate dilution of the extract was then added to 2.0 mL of ABTS solution and the absorbance was read at 732 nm after 15 mins. The TROLOX equivalent antioxidant capacity was subsequently calculated.

#### **2.4.6 Superoxide anion scavenging activity assay**

The superoxide anion radicals were produced in 2 mL of phosphate buffer (100 mM, pH 7.4) with

78 µM β-nicotinamide adenine dinucleotide (NADH), 50 µM nitro blue tetrazolium chloride (NBT) and test samples at different concentrations. The reaction mixture was kept for incubation at room temperature for 5 min. It was then added to 5-methylphenazinium methosulphate (PMS) (10 µM) to initiate the reaction and incubated for 5 min at room temperature. The colour reaction between superoxide anion radical and NBT was read at 560 nm. Gallic acid was used as a positive control agent for comparative analysis. The reaction mixture without test sample was used as control and without PMS was used as blank [30].

#### **2.4.7 Determination Fe<sup>2+</sup> Chelation**

The ability of the extract to chelate Fe<sup>2+</sup> was determined using a modified method of Minotti and Aust (1987) described by [28]. Briefly, 150 mM FeSO<sub>4</sub> was added to a reaction mixture containing 168 mL of 0.1 M Tris-HCl pH 7.4, 218 mL saline and extracted. The volume was made up 1 mL with distilled water. The reaction mixture was incubated for 5 min, before the addition of 13 ml of 1, 10-phenantroline. The absorbance was read at 510 nm [30,31].

### **3. RESULTS**

The results of the present study are depicted in Tables 1 to 13.

Table 1 shows the presence of secondary plant metabolites. It was observed that only ethanol extract of the stem has the flavonoids while alkaloids, cardiac glycosides, saponins, steroids, anthraquinones, tannins are present in the ethanol extract of the leaf, bark and root.

Table 2 shows the quantitative chemical analysis of minerals present in the ethanol extract of *M. lucida* leaf, stem and bark. Sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn) and phosphorus (P) were found in the leaf, stem and bark. Copper was not found in the leaf. Lead was not found in the leaf, stem nor in the bark.

Table 3 shows the quantity and composition of anti-nutrient in ethanol extracts of *M. lucida* in percentage. The crude leaf extracts contains phylates and flavonoids and it has the largest deposit of the anti-nutrient, which are 12.40% and 10.40% respectively. Only phylates and oxalates are found in the bark (1.25% and 1.50% respectively) and stem extracts (1.30% and 2.00% respectively).

**Table 1. Qualitative analysis of the phytochemical screening of *M. lucida***

Sample	Flavonoids	Alkaloids	Cardiac glucosides	Saponins	Steroids	Antraquinones	Phenols	Tannins
<i>M. lucida</i> leaf	-	+	+	+	+	+	+	+
<i>M. lucida</i> bark	-	+	+	+	+	+	+	+
<i>M. lucida</i> stem	+	+	+	+	+	+	+	+

Key:(-) = Negative (+) = Positive

**Table 2. Quantitative chemical analysis of minerals present in the ethanol extract of *M. lucida***

Sample	Na(%)	K(%)	Ca(%)	Mg(%)	Zn(%)	Fe(%)	Pb(%)	Cu(%)	Mn(%)	P(%)
<i>M. lucida</i> leaf	20.33	41.24*	15.33	25.38	18.71	4.38	ND	ND	15.34*	85.42**
<i>M. lucida</i> bark	20.01	26.17	31.42	22.06	25.02	5.70	ND	0.01	6.20	25.12
<i>M. lucida</i> stem	19.82	24.77	29.49	24.21	36.10	6.53	ND	0.02	5.45	35.78

Results from bark, leaf and stem were compared with one another. \* $P < 0.05$ , \*\* $P < 0.01$ , (Student's *t*-test); Key -ND =Not detected

**Table 3. Quantitative analysis of anti-nutrient present in plant extracts in percentage (%)**

Parameters	Leaf	Bark	Stem
Tannins (%)	2.25**	ND	ND
Phenols (%)	3.47*	ND	ND
Phylates (%)	12.42**	1.25	1.30
Oxalates (%)	8.59	1.50	2.00
Saponins (%)	7.60	ND	ND
Flavonoids (%)	10.40**	ND	ND
Alkaloids (%)	4.37**	ND	ND

Results from the bark, leaf and stem were compared with one another. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 (Student's t-test). ND = Not detected

Table 4 shows the proximate nutrient percentage composition of ethanol extracts of *M. lucida* contain Carbohydrates, moisture, ash, fats crude protein and fibre. It was observed that carbohydrates and fat has the highest quantity in the ethanol extract of the leaf with 53.74% and 16.19% respectively. The ethanol extract of the bark has the highest quantity of fibre, crude protein, moisture content and ash with 10.33%, 6.59%, 9.12% and 10.56% respectively.

Table 5 shows the *In-vitro* antioxidant activity of the ethanol extracts of *M. lucida*. It was observed

that the extract has phenol, ABTS<sub>μ</sub> (Azinobis,3-ethylbenthiazoline,6-sulphuric acid), Fe<sup>2+</sup>, FRAP (Ferric reducing property), DPPH (Diphenyl-2-picrylhydrazyl), H<sub>2</sub>O<sub>2</sub> (Hydrogen peroxide) and SO (Superoxide) activity in 5, 10 and 20 mg/ml concentration of the leaf, stem and bark extracts.

Table 6 represents the phenol activity of the antioxidant property of the ethanol extracts of the parts of *M. lucida*. The leaf has the highest value and the bark the least with 25.57% and 15.61% respectively.

Table 7 represents the flavonoid content of the ethanol extract of *M. lucida*. The stem has the highest value and the leaf has the lowest value with 11.63% and 8.73% respectively.

Table 8 represents the Ferric reducing property of the ethanol extract of *M. lucida*. The leaf has the highest value and the stem has the lowest value with 42.45% and 30.87% respectively.

Table 9 represents the Hydrogen peroxide content of the ethanol extract of *M. lucida*. The leaf has the highest value and the stem has the lowest value with 60.84% and 49.54% respectively.

**Table 4. Quantitative analysis of proximate nutrient composition of *M. lucida* ethanol extracts**

Samples	% Ash	% MC	% Fat	% CP	% Fibre	% CHO
<i>M. lucida</i> leaf	8.75	7.35	16.19	5.42	8.55	53.74
<i>M. lucida</i> bark	10.56	9.12	14.45	6.59	10.33	42.59
<i>M. lucida</i> stem	10.12	9.00	12.01	6.55	10.12	41.17

MC = Moisture content, CHO = Carbohydrate, CP = Crude protein

**Table 5. Antioxidant assay of ethanol extract of *M. lucida***

Concentration	Stem			Bark			Leaf		
	5 mg/ml	10 mg/ml	20 mg/ml	5 mg/ml	10 mg/ml	20 mg/ml	5 mg/ml	10 mg/ml	20 mg/ml
Phenols (%)	5.34	10.57	16.22	5.25	10.35	15.61	8.51	17.06	25.57**
Flavonoids (%)	3.35	7.67	11.63	3.18	5.69	8.73	3.71	7.70	11.16
Frap (%)	10.88	20.59	30.87	12.03	26.08	36.40	14.00	28.60	42.45**
FE <sup>2+</sup> (%)	21.84	33.52	53.26	27.01	40.54	55.65	35.54	51.44	61.69*
H <sub>2</sub> O <sub>2</sub> (%)	19.35	34.06	49.54	25.70	32.97	51.08	34.21	48.61	60.84
ABTS <sub>μ</sub> (%)	14.20	16.90	19.19	19.65	21.46	22.75	14.56	17.22	19.61
DPPH (%)	9.65	27.78	45.65	32.81	49.68	59.28	17.08	35.97	48.86
SO (%)	15.36	32.21	59.74	19.29	33.52	77.90**	30.14	49.81	61.42

Difference between bark, leaf and stem were compared with one another. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001. (Student's t-test)

Key: FRAP = Ferric reducing property (unit = %); ABTS = Azino-bis (3-ethylbenthiazoline-6-sulphuric acid) (unit = μ); SO = Superoxide; DPPH = Diphenyl -2-picrylhydrazyl (unit = %) and H<sub>2</sub>O<sub>2</sub> = (unit = %)

**Table 6. Phenol content of antioxidant property *In-vitro* of *M. lucida***

Plant Part	Phenol Activity (%)
<i>M. lucida</i> Stem ethanol	16.22
<i>M. lucida</i> Bark ethanol	15.61
<i>M. lucida</i> Leaf ethanol	25.57*
Garlic (Standard)	62.65

Difference between bark, leaf and stem were compared with one another. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . (Student's *t*-test)

**Table 7. The Flavonoid content of *M. lucida***

Plant Part	Flavonoid content (%)
<i>M. lucida</i> Stem ethanol	11.63
<i>M. lucida</i> Bark ethanol	8.73
<i>M. lucida</i> Leaf ethanol	11.16
Rutin (standard)	47.09

**Table 8. FRAP content of antioxidant *In-vitro* of *M. lucida***

Plant Part	FRAP: Ferric reducing property (%)
<i>M. lucida</i> Stem ethanol	30.87
<i>M. lucida</i> Bark ethanol	36.41
<i>M. lucida</i> Leaf ethanol	42.45*
Vitamin C (standard)	84.71

Difference between bark, leaf and stem were compared with one another. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . (Student's *t*-test)

**Table 9. H<sub>2</sub>O<sub>2</sub> *In-vitro* activity ethanolic extracts of *M. lucida***

Plant Part	Hydrogen peroxide activity (%)
<i>M. lucida</i> Stem ethanol	49.54
<i>M. lucida</i> Bark ethanol	51.08
<i>M. lucida</i> Leaf ethanol	60.83
Vitamin C (standard)	86.77

Difference between bark, leaf and stem were compared with one another. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . (Student's *t*-test).

Table 10 represents the ABTS  $\mu\text{M/g}$  % (Azinobis 3-ethylbenthiiazoline, 6- sulphuric acid) antioxidant activity of the ethanol extract of *M. lucida*. The bark has the highest value and the stem has the lowest value with 22.15% and 19.19% respectively.

Table 11 represents the Iron reducing antioxidant property of ethanolic extract of *M. lucida*. The

leaf has the highest value and the stem has the lowest value with 61.69% and 53.26% respectively.

**Table 10. ABTS antioxidant *In-vitro* activity of *M. lucida***

Plant Part	ABTS antioxidant activity ( $\mu\text{M/g}$ %)
<i>M. lucida</i> Stem Ethanol	19.19
<i>M. lucida</i> Bark Ethanol	22.15
<i>M. lucida</i> Leaf Ethanol	19.61
Trolox (standard)	92.07

**Table 11. Fe<sup>2+</sup> content of antioxidant *In-vitro* of *M. lucida***

Plant Part	Fe <sup>2+</sup> (%)
<i>M. lucida</i> Stem Ethanol	53.26
<i>M. lucida</i> Bark Ethanol	55.65
<i>M. lucida</i> Leaf Ethanol	61.69*
EDTA	86.09

Difference between bark, leaf and stem were compared with one another. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . (Student's *t*-test).

Table 12 represents the DPPH content antioxidant activity of ethanolic extract of *M. lucida*. The bark has the highest value and the stem has the lowest value with 59.78% and 45.65% respectively.

**Table 12. DPPH *In-vitro* activity of *M. lucida***

Plant Part	DPPH (%)
<i>M. lucida</i> Stem ethanol	45.65
<i>M. lucida</i> Bark ethanol	59.78*
<i>M. lucida</i> Leaf ethanol	48.86
Trolox (standard)	93.00

Difference between bark, leaf and stem were compared with one another. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . (Student's *t*-test).

Table 13 represents the Superoxide antioxidant property of ethanolic extract of *M. lucida*. The leaf has the highest value and the stem has the lowest values with 61.42% and 51.87% respectively.

#### 4. DISCUSSION

Plants used in the treatment of diseases are said to contain bioactive principles with biological activity some of which are responsible for the characteristic odor, pungencies and color of plant, while others give the particular plant its culinary, medicinal or poisonous virtue [32,33].



**Table 13. So *In-vitro* activity of *M. lucida***

Plant Part	SO (%)
<i>M. lucida</i> Stem ethanol	59.74
<i>M. lucida</i> Bark ethanol	51.87
<i>M. lucida</i> Leaf ethanol	61.42
Vitamin C (standard)	94.21

Elemental composition, evaluation of phytochemical constituents and antioxidant potential of the ethanol extract of *M. lucida* leaf, stem and bark were investigated in the present study. It was observed that the plant contained many bioactive compounds. The ethanol extracts contain flavonoids and anthraquinones which are known antioxidants [34]. Flavonoids are known to have anti-microbial, anti-ulcer, anti-virus and anti-tumor properties [35-37]. They also affect platelet aggregation. The presence of flavonoids may play a role in its folkloric use. It was however observed that the leaf contains significantly higher amount of flavonoids, alkaloids, saponins and tannins. The actions due to the presence of different trace elements play a major role in the prevention and management of some infections and communicable diseases. It may also be a source of trace elements in the prevention and management of some nutritional disorders. This is an important property of the medicinal plant; nutrients like calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), potassium (K) and phosphorus (P) play major roles in the chemical balance of human system and food preservation. Toxic lead (Pb) was not detected in all parts under investigation. The composition of phyto-constituents content (phenols, phylates, tannins, oxalates, saponin, flavonoids and alkaloids in the ethanolic extract of leaf, bark and stem of *M. lucida* were also studied. Such compounds are known to have biological activities such as free radicals scavenging and antioxidant potential which may be able to protect the body from diseases. There is also increasing proof that antioxidants derived from indigenous plants may be useful in preventing oxidative stress, thus there is increasing interest in the protective biochemical activity with natural antioxidants present in medicinal plants. However the ethanolic extract of the leaf was significantly higher in some tests for antioxidant property which may show that the leaf may have better antioxidant profile as compared with the stem and bark. The proximate analysis of the ethanol content of *M. lucida* gave the observation that the plant contains carbohydrates, crude protein, fats, fibre and Ash which supports the claim that the plant is edible and nutritious [5,38,39]. The plant

can also be of use in the food industry as it may be useful as food supplements as it may be a potent source of nutrients and trace elements. It is quite interesting to inform that these plant metabolites can be genetically manipulated to increase their yield. Additionally, the DNA copy or gene(s) responsible for the expression of these metabolites could be cloned and inserted into other edible crops for ease of consumption by end users. This implies that it might be unnecessary to go over the counter medicine stores to buy synthetic drugs to this respect.

## 5. CONCLUSION

This research work presented demonstrated that the ethanolic extracts of leaf, stem and bark of *M. lucida* have antioxidant activity and is nutritious due to its chemical composition. It also investigated the phytochemical constituents of the ethanolic extract. The current findings are a step forward to increasing knowledge and usefulness of *M. lucida* as a medicinal plant. It can be useful as a food supplement.

## 6. RECOMMENDATION

It is thereby recommended that medicinal plants such as *M. lucida* should be studied and exploited for future use.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist

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