

Evaluation of Bioactive Compounds, in Vitro Antioxidant Activity and Acute Toxicity of Ethanol Extract of *Morinda lucida* Leaves

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Authors' contributions

This work was carried out in collaboration among all authors. Author FMI designed the study, wrote the protocol, performed the statistical analysis, and wrote the first draft of the manuscript. Authors OVA and JE managed the analyses of the study. Author VDS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Plants are known to contain phytochemicals of pharmacological relevance and as such have been utilized in the treatment and management of various diseases. *Morinda lucida*, a medium size tropical tree belonging to the rubiaceae family and widely distributed in Africa is one of these plants. It has been reportedly used in the traditional treatment and management of diseases. This study is aimed at identifying compounds with pharmacological relevance in the ethanol extract of *Morinda lucida* leaves, the antioxidant activity and lethal dose determination of the extract. The leaves of *Morinda lucida* was extracted with ethanol; phytochemical and bioactive compounds analysis, in vitro antioxidant activity and lethal dose (LD₅₀) determinations were carried out. It was observed in the study that the extract contains alkaloids, quinines, quinones, flavonoids and tannins. The gas

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chromatography mass spectrometry (GC-MS) identified phenol 2, 4-bis (1,1-dimethylethyl) (2.82%), Stilbenes (12.32%), Phenoxazine (2.60%) and Benz(cd) indol-2(1H)-one, 1-methyl- (2.60%) amongst other compounds in the extract. The in vitro antioxidant activity evaluation of the extract revealed that it possesses a significant antioxidant activity which increased with increasing concentration. The LD₅₀ determination revealed the extract was safe as there was no death recorded even at a dose as high as 5000 mg/kg. This study shows that *Morinda lucida* possesses enormous pharmacological potentials.

Keywords: *Morinda lucida*; bioactive compounds; GC-MS; antioxidant; phytochemicals; LD₅₀.

1. INTRODUCTION

The use of medicinal plants has been recorded throughout human history. Plants have the capability to synthesize wide range and varieties of phytochemicals that have the ability to exert important biological functions. Some of these phytochemicals have been widely researched and confirmed to possess medicinal properties. Medicinal plants continue to play important roles in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care [1].

Morinda lucida, a medium size tropical tree of about 15 meters tall having a scaly grey bark with short crooked branches and shining foliage. It belong to the *Rubiaceae* family; flowering plants variously called the madder family, bedstraw family or coffee family. The family takes its name from the madder genus *Rubia*. The plants included in the family are *Cofea arabica*, *Morinda lucida*, *Cinchona* (whose bark contains quinine). According to Lawal et al. [2] Its generally used as ingredients of fever teas, which are usually taken, for the traditional treatment of malaria. In West Africa *Morinda lucida* is an important plant in traditional medicine. In Africa, *M. lucida* is found mainly in West Africa and Central Africa. In the West Africa region *M. lucida* is spread across the coastal countries including Nigeria [3]. *Morinda lucida* is one of the four most used plants in the preparation of traditional medicines against fever in Nigeria. Decoctions and infusions or plasters of root, bark and leaves are recognized remedies against different types of fever, including yellow fever, malaria, trypanosomiasis and feverish condition during childbirth. The boiled leaves of *M. lucida* is used for the treatment of malaria in some part of Nigeria especially in the north central geopolitical zone where other parts of the plant such as the bark is also used for the treatment of meningitis. The leaves have also been reported to possess strong hypoglycemia, trypanocidal and aortic vasorelaxant activities [4].

This study is aimed at identifying the phytochemicals and bioactive compounds in the ethanol extract of *Morinda lucida* and to determine its in vitro antioxidant activity as well as the acute toxicity.

2. MATERIALS AND METHODS

2.1 Sample Collection

The leaves of *Morinda lucida* were collected from Kogi State University Campus Anyigba, Kogi State, Nigeria and identified in the Botany Unit of the Department of Biological Sciences, Kogi State University, Anyigba, Kogi State, Nigeria with Batch Number 050.

2.2 Experimental Animals

Twelve (12) Albino Mice with average weight of 25 g were purchased from the experimental animal section of Salem University, Lokoja, Kogi State.

2.3 Sample Preparation and Extraction

The leaves of *Morinda lucida* were air dried and pulverized using a Binatone BLG-450 blender. The pulverized sample was extracted with ethanol using the soxhlet extraction method. The extracted sample was weighed into a thimble and loaded into the chamber of the soxhlet extractor; 150 ml of the solvent was poured into a different chamber attached to the soxhlet extractor, enough to siphon at least twice into the flask. The temperature of the experiment was set up to 75°C and heated at reflux. This cycle was repeated over again for six hours. The extract was dried at room temperature and stored at 4°C for further usage.

2.4 Qualitative Phytochemical Analysis

The qualitative phytochemical analysis was done following standard methods [5,6].

2.5 Bioactive Compounds Determination Using GC-MS

GC-MS analysis of the extract was done using a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2µl was employed (a split ratio of 25:1). The injector temperature was maintained at 250°C, the ion-source temperature was 200°C, the oven temperature was programmed from 60°C (isothermal for 2 min), with an increase of 30°C/min to 120°C, ending with a 3-min isothermal at 290°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 700 Da. The solvent delay was 0 to 2 min, and the total GC-MS running time was 21 min.

2.6 In Vitro Antioxidant Assay

2.6.1 1, 1-diphenyl 1-2-picrylhydrazyl (DPPH) radical scavenging activity

One mL of various concentrations of the extracts in methanol was added to 4 mL of 0.1 mmol L⁻¹ methanolic solution of DPPH. A blank probe was obtained by mixing 4 mL of 0.1 mmol L⁻¹ methanolic solution of DPPH and 200 µL of deionized water. After 30 minutes of incubation in the dark at room temperature, the absorbance was read at 517 nm against the prepared blank. Inhibition of free radicals by DPPH in percent (%) was calculated using this formula: %Inhibition = (ABSsample – ABS blank)/ ABS control) x100

2.7 Lethal Dose (LD₅₀) Determination

The LD₅₀ was determined using Lorke's methods [7], the LD₅₀ was calculated as the geometric mean of the highest non-lethal dose (with no deaths) and lowest lethal dose (where death occurred).

The experimental animals were observed for signs of toxicity and mortality, they were observed individually at least once during the first 30 minutes, with special attention during the first 4 hours after dosing. The animals were placed under observation for 24 hours after administration and allowed access to food and water at libitum to check acute and sub-acute toxicity through record of mortality and other loss of vital visible functions.

3. RESULTS

3.1 Phytochemical Screening

Table 1 shows some of the phytochemicals detected in the ethanol extract of *Morinda lucida* leaves which are known to possess pharmacological properties.

3.2 Gas Chromatography Mass Spectroscopy

Table 2 and Fig. 1 represent the result from the GC-MS analysis of the ethanol extract of *Morinda lucida*. The result showed the extract contains some compounds of pharmacological relevance.

3.3 In Vitro Antioxidant Activity

Fig. 2 shows the in vitro antioxidant properties of the extract. The extract showed significant antioxidant activity which increased with increasing concentration of the extract.

Table 1. Phytochemical screening of ethanol extract of *Morinda lucida* leaves

Phytochemicals	Results
Alkaloids	++
Quinines	++
Quinones	+
Anthraquinons	+
Tannins	+
Flavonoids	++
Saponins	+
Glycosides	+
Terpenoids	-

(-) = not present

(+) = present

(++) = conspicuously present

Table 2. Compounds present in ethanol extract of *Morinda lucida* leaves

Peak #	R.T. min	Fist scan	Max scan	Last scan	PK TY	Peak height	Corr. area	Corr. % max.	% of total	Area%	Library/ID
3	18.589	262	267	278	rBV	6516	65873	8.97%	2.823%	2.82	Benzeneacetic acid, 4-(1,1-dimethylethyl)-, methyl ester 2,4-bis(1,1-dimethylethyl) Phenol Oxirane, [[4-(1,1-dimethylethyl)phenoxy] methyl]-
6	25.593	394	405	433	rBV	7917	287546	39.13%	12.323%	12.32	(E)-Stilbene (E)-Stilbene Phenanthrene, 9,10-dihydro-
18	60.768	1094	1098	1112	rVB3	1996	60577	8.24%	2.596%	2.60	Phenoxazine Benz(cd) indol-2(1H)-one, 1-methyl- 2-Amino-1-acenaphthenone

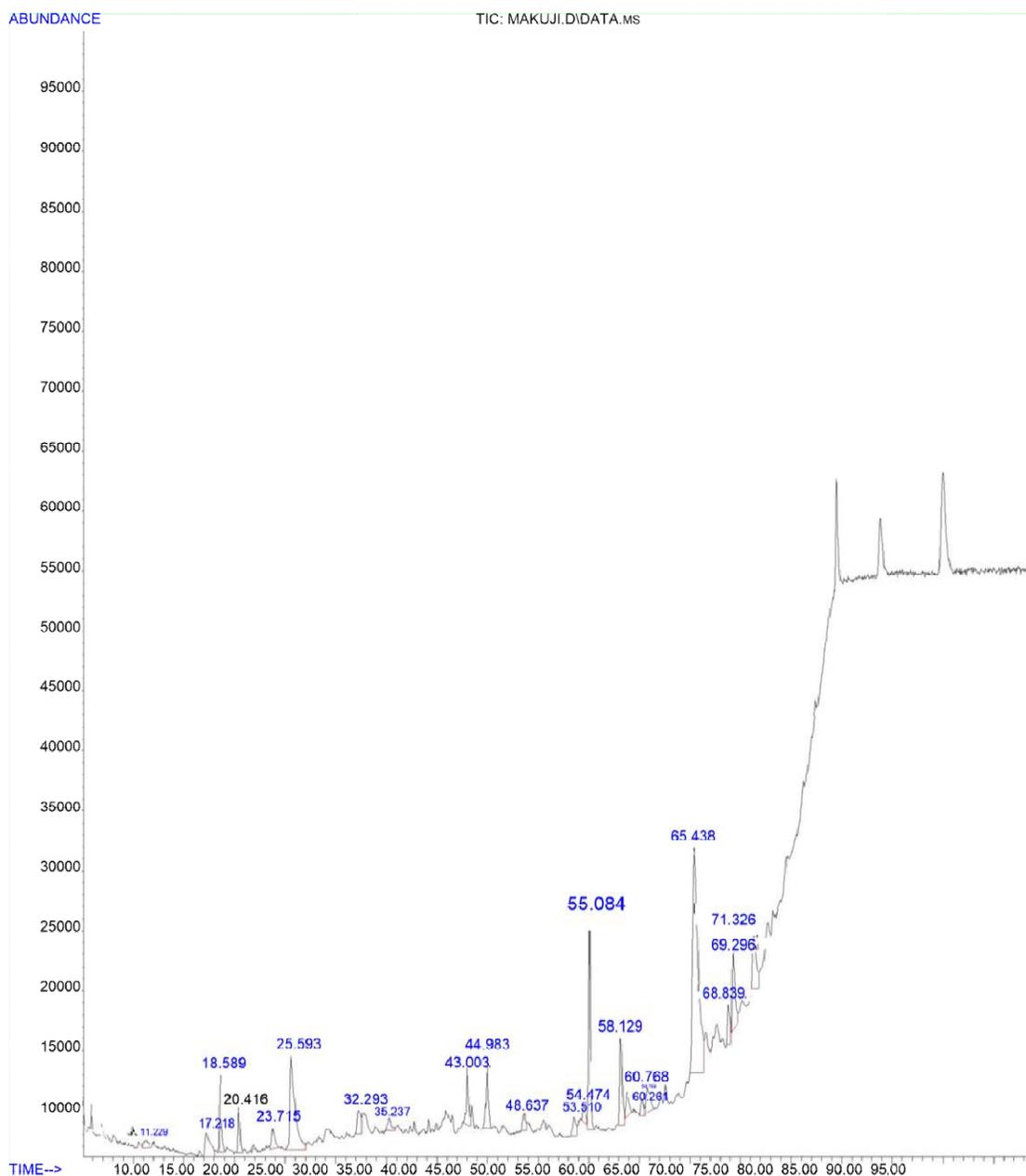


Fig. 1. GCMS spectrum of ethanol extract *Morinda lucida* leaves

Table 3. Lethal dose (LD₅₀) of the ethanol extract of *Morinda lucida* leaves

Doses	Number of Deaths Recorded	Observation In Physical Appearance
10 mg/kg	NIL	Normal
100 mg/kg	NIL	Normal
1000 mg/kg	NIL	Normal
1600mg/kg	NIL	Normal
2900mg/kg	NIL	Normal
5000mg/kg	NIL	Normal

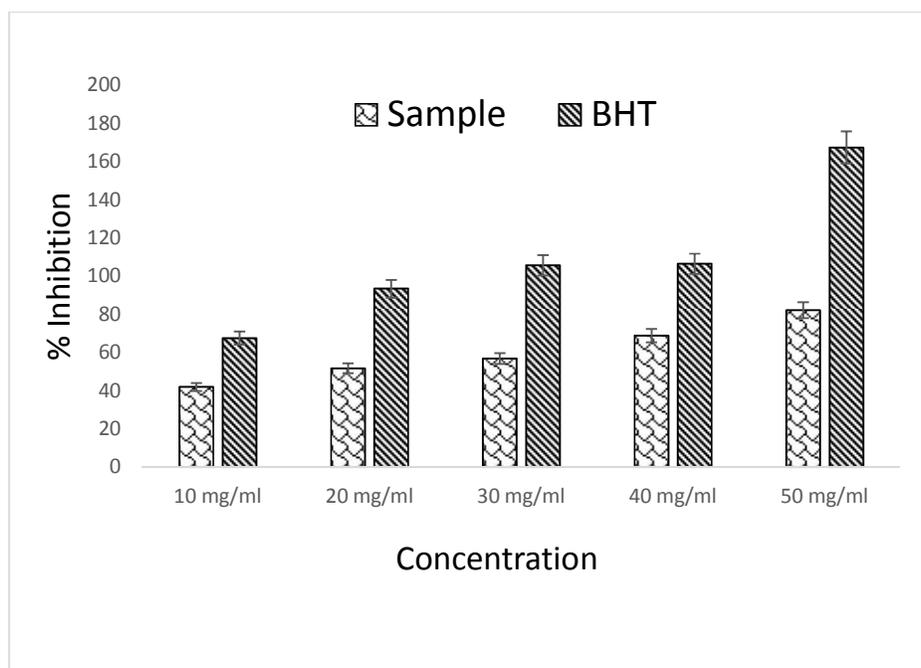


Fig. 2. 1-diphenyl 1-2-picrylhydrazyl (DPPH) radical scavenging activity of ethanol extract of *Morinda lucida* leaves

4. DISCUSSION

Plants are known to contain chemical compounds which are generally known as phytochemicals. Some of these compounds possess pharmacological activities as well as other bioactive potentials. The use of plants for various purposes beneficial to humans especially in the treatment of ailments dates back to many centuries ago. *Morinda lucida* is one of these plants with numerous pharmacological benefits.

Researching into bioactive compounds in plants especially those known for their traditional usage in the treatment of disease is important as there is rise in number of diseases around the globe and resistance of some diseases to existing treatment options.

In this study it was observed that the ethanol extract of *M. lucida* leaves possess phytochemicals of pharmacological relevance (Table 1), these include alkaloids, quinines, quinones, flavonoids and tannins. Further analysis using gas chromatography mass spectroscopy identified the compounds found in the extract (Fig. 1 and Table 2); these include amongst others phenol, 2,4-bis(1,1-dimethylethyl) (2.82%) a compound which was reported in a study by Padmavathi et al. [8] to

inhibit and disrupt biofilms as well as increasing the susceptibility of *S. marcescens* to gentamicin when administered synergistically. The study suggested that this opened another avenue for combinatorial therapy where phenol, 2,4-bis(1,1-dimethylethyl) can be used to enhance the efficacy of conventional antibiotics; Stilbenes (12.32%) were also found in the extract, this compound according to Alberta et al. [9] inhibits zones for bacteria and fungi in a way that was comparable with those of the antibiotics tetracycline, streptomycin, ampicillin, or kanamycin, directed against prokaryotes, and nourseothricin or hygromycin controlling fungi, respectively; Phenoxazine (2.60%) a compound reported by Flanagan et al. [10] to enhance cellular penetration, nuclear accumulation, and subsequent antisense activity was found in the extract and Benz(cd) indol-2(1H)-one, 1-methyl-(2.60%) involved in treating, inhibiting or controlling a RAS-associated disease by inhibiting farnesyl-protein transferase(FPTase) enzyme, treating RAS oncogene dependent tumors according to Ayril-Kaloustian et al. [11] was also present in the extract.

The in vitro antioxidant activity evaluation of the extract revealed that it possessed a significant antioxidant activity which increased with increasing concentration of the extract. This

validates the use of this plant in the management and prevention of diseases related to oxidative stress such as diabetes and cancer.

Further study was carried out to determine the LD₅₀ of this extract, the result showed the extract was safe as there was no death record even at a dose as high as 5000mg/kg. This study suggest further research into possibility of harnessing the enormous pharmacological potential of *Morinda lucida*.

5. CONCLUSION

This study revealed the ethanol extract of *Morinda lucida* leaves contain compounds of pharmacological relevance as well as those relevant in other biological activity, this compounds include benz (cd) indol-2 (1H)-one, 1-methyl-, Phenoxazine, stilbenes and phenol, 2,4-bis(1,1-dimethylethyl) amongst others. The extract also possess significant antioxidant activity. This study suggest further research into possibility of isolating pure compounds with pharmacological relevance from *Morinda lucida*.

ETHICAL APPROVAL

The animals were acclimated in the experimental room for 2 weeks and were handled according to the guidelines on the use of experimental animals by the Ethical Committee of Kogi State University Anyigba, Kogi State, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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