

Assessment of antioxidant properties of N-Hexane extract of *Morinda lucida* as a link to its pharmacological actions

Abstract

Background and objective: *Morinda lucida* is a flowering plant which belongs to the family, Rubiaceae and reported to possess numerous pharmacological properties. This study was carried out to assess the antioxidant potentials of n-hexane extracts of *M. lucida* as a link to its medicinal actions.

Methods: The leaves of *M. lucida* were collected from the local suppliers and the n-Hexane extract of the leaves was prepared using Soxhlet extractor. The extract was then concentrated in a rotary evaporator and investigated for different antioxidant parameters.

Results: The results of the study revealed that n-hexane *M. lucida* possess substantial radical scavenging ability, ferric reducing power, iron chelating ability and antilipid peroxidation property compared to the control ($P < 0.05$). The documented medicinal properties of the plant can therefore be attributed to the experimentally proved and ascertained antioxidant action.

Keywords: antioxidants, *Morinda lucida*, n-hexane extract, lipid peroxidation, reactive oxygen species

Introduction

Reactive oxygen species (ROS) are a class of highly reactive molecules that can cause damage to cells and tissues during various infections and degenerative disorders including cancer.¹ ROS occur in tissues participating in potentially deleterious reactions (Paolo *et al.*, 1991) and are capable of damaging DNA, proteins, carbohydrates and lipids in the tissues.² ROS include superoxide anion, hydrogen peroxide, hydroxyl radical and singlet molecular oxygen. A free radical is any chemical species that can exist independently and possesses one or more unpaired electron.^{3,4} Lipid peroxidation mediated by free radical is considered to be primarily responsible for cell membrane destruction and cell damage leading to tissue injury and failure of the antioxidant defence mechanism (Comporti, 1989).

An antioxidant is defined as a molecule capable of slowing or preventing the oxidation of other molecules. Antioxidants acts as radical scavenger and inhibit lipid peroxidation and other free radical-mediated processes, thereby protecting the human body from various diseases (Czinner *et al.*, 2001). Natural antioxidants, such as vitamin E (tocopherol), vitamin C, and polyphenols/flavonoids, have been investigated for their possible use to prevent different diseases.⁵ Recently, polyphenols/flavonoids found in plants are now being investigated and considered by researchers as a new natural antioxidant. Consequently, numerous reports on natural antioxidants, including polyphenols, flavonoids, vitamins, and volatile chemicals have been reported.⁶

Morinda is a genus of flowering plants in the madder family, Rubiaceae.⁷ *Morinda lucida* is a tropical West Africa rainforest tree also called Brimstone tree. In South-Western Nigeria, it is called Oruwo.^{7,8} It is a medium sized tree at maturity. Different parts of *M. lucida* have been reported to possess medicinal properties. The leaf extract of the plant was reported to possess trypanocidal,⁹ antimarial activities^{10,11}

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and aortic vasorelaxant effect.¹² The weak decoction of the stem bark has been documented to possess anti-jaundice property.¹³ Studies also revealed that *M. lucida* leaf extract possess strong oral hypoglycemic property.^{7,14} The leaf extract of *M. lucida* has also been documented to possess anti-spermatogenic activities in rats.¹⁵ Its stem bark infusion is used as antimalarial and antidiabetic agent. It showed significant antimalarial activity^{9,10,11,16-20} and anti-*Salmonella typhi* activity.¹⁸ *M. lucida* extracts induced contractility effect on isolated uterine smooth muscle of pregnant and non-pregnant mice.^{11,12} The root of *M. lucida* is used as chewing sticks 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*.²¹⁻²³ This present study sought to assess the antioxidant potentials of n-hexane extracts of *M. lucida* as the rationale behind its myriads of pharmacological activities.

Methods

Sample collection

The leaves of *M. lucida* were collected from the local suppliers in Akure, Ondo state (Nigeria). The plants collected were identified botanically in Department of Biological Science, Federal University, Wukari. Fresh and tender leaves of selected plants were air-dried for two weeks at 25°C. The dried leaves were powdered with mechanical grinder to obtain coarse powder and used for antioxidant assays.

Chemicals

Potassiumpersulphate ($K_2S_2O_8$), 2,2'-azinobis-3 ethylbenzothiazoline-6-sulfonic acid (ABTS), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), iron (II) sulphate, thiobarbituric acid (TBA) were obtained from Sigma (St. Louis, MO). All chemicals used were of analytical grade and were obtained from standard commercial suppliers.

Preparation of n-Hexane extract

The n-Hexane extract of *M. lucida* was prepared using Soxhlet extractor. 50.0g of pulverized leaves was put into thimble and boiled in n-hexane on a heating mantle at the temperature of 68°C. The hexane boiled and washed the extract into the round bottom flask. It was allowed to siphon six (6) times. The n-hexane extract was then concentrated in a rotary evaporator and the n-hexane used for the extraction was recovered. The dried extract was stored in refrigerator for further use.

Antioxidants assays

Determination of ABTS radical scavenging ability

Antioxidant activity of the extract was determined using the 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid(ABTS) antiradical assay.²⁴ The ABTS⁺ (working solution) was prepared by mixing equal volumes of 7mM ABTS and 2.45mM potassium persulphate ($K_2S_2O_8$) (both prepared using distilled water) in a volumetric flask, which was wrapped in foil and allowed to react for a minimum of 12hrs in the dark. The working solution (900 μ l) was added to the n-hexane extracts (300 μ l). The test tubes were allowed to stand for exactly 30min. The absorbance of the standards and samples was read at 700nm in a spectrophotometer.

1,1-diphenyl-2 picrylhydrazyl (DPPH) radical scavenging ability

The free radical scavenging ability of the extracts against DPPH free radical was evaluated as described by Gyamfi et al.²⁵ Briefly, five concentrations (1ml) of the extract were added to 1ml of 0.2Mm methanolic solution containing DPPH radicals, the mixture was left in the dark for 30mins and the absorbance was taken at 517nm. The DPPH free radical scavenging ability was then calculated.

Determination of ferric reducing antioxidant power (FRAP)

The reducing power of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution in the presence of TPTZ as described by Nair et al., (2007). 100 μ l aliquot extracts was mixed with FRAP solution containing 100ml of 30mM acetate buffer, 10ml of 10mM TPTZ, 40mM HCl and 10ml of $FeCl_2$. The mixture was incubated at 37°C for 30min, and the absorbance was measure at 593nm and ferric reducing power was calculated.

Fe²⁺ Chelating assay

The Fe²⁺ chelating ability of the extract was determined using a modified method of Puntel et al.²⁶ 168 μ l 0.1M Tris-HCl (pH 7.4), 218 μ l of Saline and extract were added to freshly prepared 500 μ M $FeSO_4$ (150 μ l). The reaction was incubated for 5min followed by addition of 13 μ l 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510nm in a spectrophotometer. The Fe²⁺ chelating ability was subsequently calculated as percentage (%).

Thiobarbituric acid reactive species (TBARS) assay

Rat was decapitated under mild ether anaesthesia and the liver was quickly removed, placed on ice and homogenized in cold 5mM Tris-HCl pH 7.4. The homogenate was spinned at 4000r/m for 10min in cold centrifuge to yield a low speed supernatant fraction that was used for lipid peroxidation assay. The lipid peroxidation was carried out by measuring TBARS (thiobarbituric acid reactive species) using the modified method of Ohkawa et al.²⁷ An aliquot of 100 μ l of homogenate was incubated for 1hour at 37°C in the presence of extract (final concentrations range of 0-100 mg/ml), in a reaction

system containing 50 mM Tris-HCl buffer (pH 7.4), 10 μ M $FeSO_4$ (prooxidant). The colour reaction was developed by adding 200 μ l of 8.1% SDS (Sodium Dodecyl Sulphate) to the reaction mixture, this was subsequently followed by the addition of 500 μ l of acetate buffer, pH 3.4, and 500 μ l of 0.8% TBA. This mixture was incubated at 100°C for 30 minutes. TBARS produced was measured at 532 nm in UV-visible spectrophotometer.

Results

ABTS radical scavenging ability

The result in figure 1 reveals that the extract reduced ABTS radical by scavenging it in concentration dependent manner. This scavenging ability is significant ($P<0.05$) when compared with the control, with the highest concentration having the least amount of unscavenged radicals.

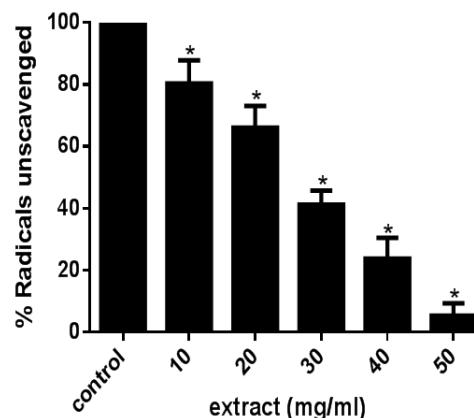


Figure 1 ABTS radical scavenging abilities of extracts of *M. lucida*. Data are presented as mean \pm SEM of three independent experiments. *Represent significant difference from control at $p<0.05$.

1,1-diphenyl-2 picrylhydrazyl (DPPH) radical scavenging ability

Figure 2 shows that at concentration above 10mg/ml, the extract significantly ($P<0.05$) reduced DPPH[·] in the reaction medium in concentration dependent manner as observed from the decolorization of the purple colour of DPPH. This indicates that the extract possessed an appreciable DPPH[·] scavenging ability.

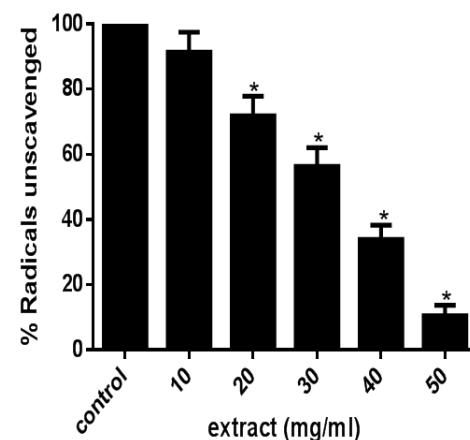


Figure 2 DPPH radical scavenging abilities of *M. lucida*. Data are presented as mean \pm SEM of three independent experiments. *Represent significant difference from control at $p<0.05$.

Ferric reducing antioxidant power (FRAP)

As revealed in figure 3, the extract significantly ($P<0.05$) reduced Fe^{3+} to Fe^{2+} at concentration of 10mg/ml upward as observed from the formation of intense blue colouration. This indicates that the extract possessed a perceptible ferric reducing ability.

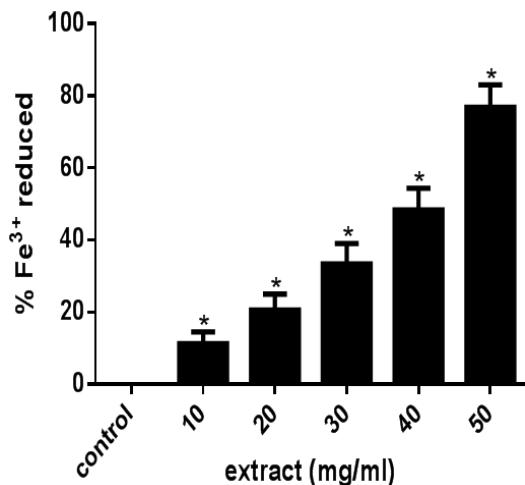


Figure 3 Ferric reducing antioxidant power of *M. lucida*. Data are presented as mean \pm SEM of three independent experiments. *Represent significant difference from control at $p<0.05$.

Iron chelating property

The Fe^{2+} chelating ability of the extract is presented in figure 4. The result reveals that the extract possess a potent ($P<0.05$) Fe^{2+} chelating ability compared to the control.

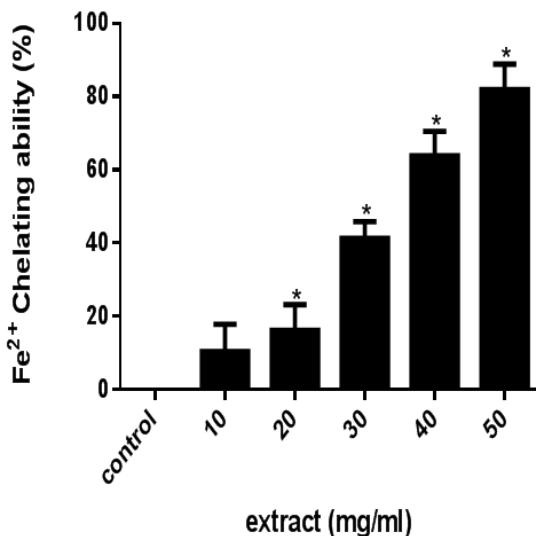


Figure 4 Fe^{2+} chelating ability of *M. lucida*. Data are presented as mean \pm SEM of three independent experiments. *Represent significant difference from control at $p<0.05$.

Effect of n-hexane extracts TBARS production

As shown in figure 5, the extract inhibited Fe^{2+} -induced lipid peroxidation in rat liver homogenate and the inhibition was significant ($P<0.05$) when compared to the control. This indicates that the extract potently prevents the formation of TBARS in rat liver homogenate.

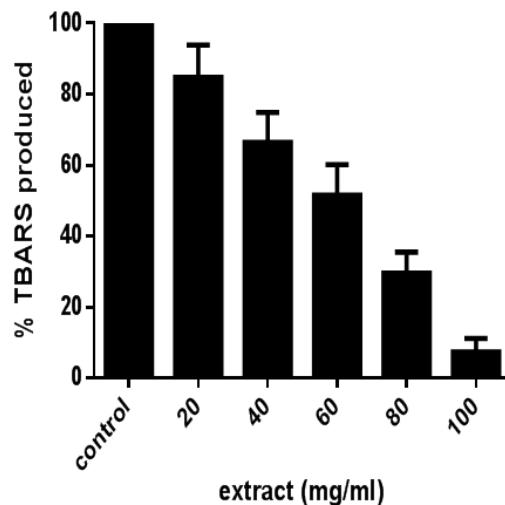


Figure 5 Effect of *M. lucida* on Fe^{2+} induced lipid peroxidation. Data are presented as mean \pm SEM of three independent experiments. *Represent significant difference from control at $p<0.05$.

Discussion

Reactive oxygen species (ROS) are a class of highly reactive molecules that can cause damage to cells and tissues during various infections and degenerative disorders including cancer.¹ Due to the critical role of scavenging free radicals including ROS by antioxidants, this property in medicinal plants is considered very relevant. *M. lucida* is a neutraceutical plant used locally for a variety of pharmacological actions such as anticancer, hepatoprotective, cytotoxic, and genotoxic, anti-spermatogenic, hypoglycemic and anti-diabetic activities.²⁸ Herein, different antioxidant parameters were carried out to shed light on the antioxidant efficacy of *M. lucida* as a link to its reported medicinal values.

The ABTS assay requires that 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) which on treatment with sodium/potassium persulphate²⁹ or MnO_4^- ³⁰ give a bluish-green radical cation (ABTS⁺). The radical cation is reduced in presence of hydrogen donating antioxidants.^{30,31} The result in figure 1 reveals that the extract reduced ABTS radical by scavenging it in concentration dependent manner with the highest concentration having the least amount of unscavenged radicals.

Moreso, DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a stable radical owing to stabilization by delocalization on to aromatic rings. DPPH[·] can trap other radicals easily but does not dimerize. Because a strong absorption band is centered at about 515 nm, the solution of DPPH radical form a deep violet colour and it becomes colorless to pale yellow when reduced upon reaction with hydrogen donor. In similar manner to that of the ABTS assay, the result of the DPPH assay (figure 2) shows that at concentration above 10mg/ml, the extract significantly ($P<0.05$) reduced DPPH[·] in the reaction medium in concentration dependent manner. This indicates that the extract possessed an appreciable DPPH[·] scavenging ability.

Moreover, FRAP assay uses antioxidants as reductant in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess. At low pH (3.6), reduction of ferric tripyridyl triazine (Fe^{3+} -TPTZ) complex to ferrous form

(which has an intense blue colour) can be monitored by measuring the change in absorption.³² As revealed in figure 3, the extract significantly ($P<0.05$) reduced Fe^{3+} to Fe^{2+} at concentration above 10mg/ml indicating that the extract possessed a perceptible ferric reducing ability.

Furthermore, another mechanism of antioxidant action is chelation of transition metals preventing catalysis of hydroperoxide decomposition and Fenton type reactions.^{33,34} In the presence of chelating agents, complex formation is disrupted leading to colour reduction. Measurement of colour reduction allows the estimation of the chelating activity of the coexisting chelator. Fe^{2+} possess the ability to move single electron by virtue of which it can allow the formation and propagation of many radical reactions.^{35,36} Figure 4 reveals that the extracts possess high Fe^{2+} chelating ability compared to the control.

Moreover, lipid peroxidation, a well-established mechanism of cellular injury in plants and animals, is an indicator of oxidative stress in cells and tissues. Lipid peroxides are unstable and decompose to form a complex series of compounds including reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) upon decomposition and their measurements are indicator of lipid peroxidation. Lipid peroxidation assay measures the inhibition of production of thiobarbituric acid reactive substances (TBARS) from sodium benzoate under the influence of the free oxygen radicals derived from Fenton's reaction. The reactive oxygen species degrade benzoate, resulting in the release of TBARS. Antioxidants from the added sample cause suppression of the production of TBARS.³⁷ As shown in figure 5, the extract inhibited lipid peroxidation in rat liver homogenate and the inhibition was significant ($P<0.05$) when compared to the control. This indicates that the extract impedes the formation of TBARS in rat hepatocytes.

Worth mentioning is the fact that medicinal plants contain varieties of bioactive compounds that account for their therapeutic values. Bioactive compounds are known to have different biological activities including free radical scavenging and antioxidant potential.^{4,9,38} Studies have revealed that the n-hexane extract of the leaves of *M. lucida* is rich in phytochemicals such as flavonoids, phenolics, tannins, saponins, steroids and cardiac glycosides.³⁹ Research by⁴⁰ showed that the presence of flavonoids in the plants may contribute to their antioxidant activity, since flavonoids have been reported to possess strong antioxidant activity. Flavonoids have also been documented to be anti-allergies, antiinflammatory, free radical scavengers, hepatoprotector, anti-microbial, anti-ulcer, anti-viral and affecting platelet aggregation.^{41,42} Flavonoids are also known to protect the vascular system and strengthen the tiny capillaries that carry oxygen and essential nutrients to all cells. It is on this note, rational to attribute the observed antioxidant properties of *M. lucida* herein to the reported bioactive compounds present in the plant. Previous reports^{26,43} also revealed that *M. lucida* contains alkaloids which confer its effectiveness against pathogenic microorganism. The plant was also reported to be a domicile of several phenols^{26,41,44} which are modifiers in the pathways of prostaglandin and thereby protect against the clumping of platelets,^{45,46} they also have the ability to block the uptake of cholesterol and facilitate its excretion from the body. It is therefore evident in this research that the reported therapeutic properties of *M. lucida* can be attributed to the observed antioxidant actions conferred on it by the resident bioactive compounds.^{47,48}

Conclusion

This study revealed that n-hexane *M. lucida* possesses strong radical scavenging ability, ferric reducing power, iron chelating ability as well as substantial antilipid peroxidation property. The documented medicinal properties of the plant can therefore be linked to its antioxidant action. Further research is needed to evaluate the efficacy *M. lucida* in the treatment and management of oxidative stress-linked diseases such as inflammations, tumors, cancers and diabetes.

Acknowledgments

None.

Conflicts of interest

Author declares that there is no conflict of interest.

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