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Comparison of the *in vitro* Antioxidant and Reducing Activities of Crude *Morinda lucida* Root Extracts

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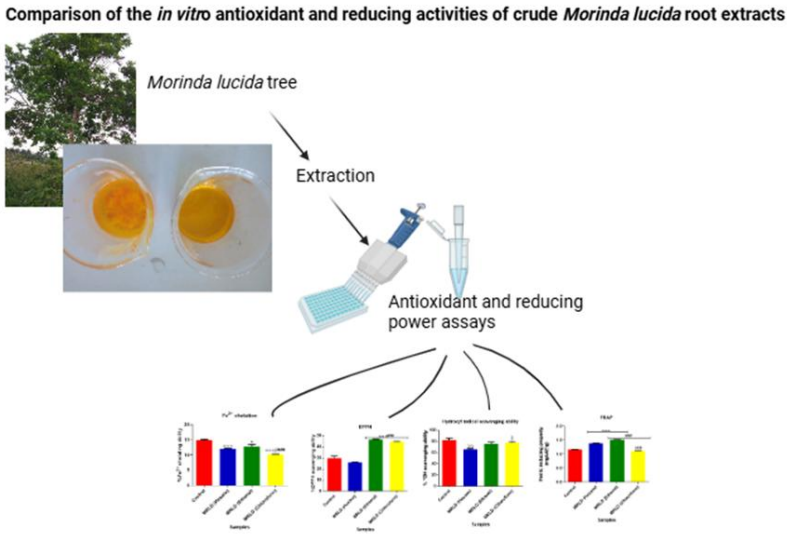
ABSTRACT

In Nigeria, one of the useful medicinal plants is *Morinda lucida.* It is widely used for medicinal purposes in Nigeria. The antioxidant and reducing properties of the crude hexane, ethanol and chloroform extracts of *Morinda lucida* were analyzed using the standard procedures for DPPH radical scavenging, ferric reducing antioxidant power (FRAP), iron chelation and hydroxyl radical scavenging were carried out using kaempferol as control. The results of this study revealed that the ethanolic extract of *Morinda lucida* significantly scavenged ($P < 0.001$) DPPH radicals when compared with the kaempferol control, the hexane and chloroform extract of *Morinda lucida*. Similarly, ethanolic extract of *Morinda lucida* significantly ($P < 0.001$) possessed the highest ferric reducing antioxidant property (FRAP) when compared with the ascorbic acid standard and other extracts of *Morinda lucida* tested. The most promising iron chelating ability was observed in the ethanolic extract of *Morinda lucida* when compared with other extracts of *Morinda lucida* tested, though the kaempferol control significantly ($P < 0.001$) had the best Fe^{2+} chelating ability. Finally, the ethanol and chloroform extracts of *Morinda lucida* significantly ($P < 0.05$) scavenged OH* radicals with no significant difference when compared with the control, while the hexane extract of *Morinda lucida* showed the lowest OH* radical scavenging ability. The assays conducted were concentration dependent with varying antioxidant potentials, hence, *Morinda lucida* could be used in the management to many forms oxidative stress related diseases, buttressing its use in folklore medicine.



KEYWORDS: Kaempferol; Chelating ability; Antioxidants; Scavenging capacity; Reactive oxygen species.

1. INTRODUCTION

The search for natural antioxidants is very important due to their potential applications in health and in food preservation. *Morinda lucida*, is a member of the Rubiaceae family, widely recognized for its extensive use in traditional medicine across various African cultures. [1-2] The roots of this plant are particularly noted for their potential therapeutic properties which are crucial in combating oxidative stress and related diseases. Recent scientific investigations have highlighted the bioactive compounds present in *Morinda lucida*,

revealing a rich phytochemical profile that includes flavonoids, alkaloids, and phenolic compounds, all of which contribute to its medicinal efficacy. [3]

The significance of antioxidants in human health cannot be overstated, as they play a vital role in neutralizing free radicals and preventing cellular damage. [4] These radicals have been identified to increase the chances of various degenerative diseases and aging processes [5]. The reducing power of a compound is often correlated with its antioxidant capacity, making it an important parameter in assessing potential antioxidant agents.

The aim of this study is to compare the *in vitro* antioxidant and reducing potentials of *Morinda lucida* roots extracts, employing various assays to quantify their effectiveness. By elucidating the antioxidant potential of these extracts, this research seeks to provide insights into their pharmacological applications.

2. MATERIALS AND METHODS

2.1 Sample Collection

Morinda lucida roots was collected from Iwo Osun state, Nigeria, the roots air dried then pulverized and extracted with three solvents, hexane, chloroform and ethanol.

2.2. Chemicals and Reagents

In this investigation, analytical-grade chemicals and reagents were used. The following products were bought from Chemie GmH (Steinheim, Germany) and Sigma Aldrich, through a chemical vendor in Nigeria. Iron sulfate ($FeSO_4$), hydrogen peroxide (H_2O_2), deoxyribose, potassium ferricyanide (KFeCN), trichloroacetic acid (TCA), iron chloride ($FeCl_3$), Naphtyl ethylenediamine dihydrochloride (NEDD), 2,4-dinitrophenyl hydrazine (DNPH), and 1'1-diphenyl-2-picryl hydrazine (DPPH).

2.3 Antioxidant Parameters

1'1-Diphenyl-2-pycryl hydrazine (DPPH) test of the plant extracts was carried out as previously described Oboh[6]. The value was calculated by plotting inhibition percentages against concentrations of the plant extracts. For Reducing power, various concentrations of the extracts in 0.5 mL samples were mixed with 1 mL of a phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1% potassium hexaferricyanide [$K_3Fe(CN)_6$], and the mixture was incubated at 50^{0}C for 30 min. Afterwards, 1 mL of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000× g for 10 min. Finally, 1 mL of the upper layer of the solution was mixed with 0.2 mL of 0.1% $FeCl_3$, the mixture was left to rest away from light and the absorbance was measured at 700 nm. The same operation was realized with BHT (0–100 µg/mL) used as a reference[7]. The antioxidant activity linked to reducing power was expressed as antioxidant power (AP) following the formula:

$$\text{AP} = \frac{Abs\ extract - Abs\ blank}{Abs\ extract}\ x\ 100.$$

The plant extracts (0.15-0.6 mM) were added to a reaction mixture containing 120 mL of 20 mM deoxyribose, 400 mL of 0.1 M phosphate buffer, 40 mL of 500 mM $FeSO_4$, and the volume were made up to 800 mL with distilled water. The reaction mixture was incubated at 37^{0}C for 30 minutes and the reaction was then stopped by the addition of 0.5 mL of 28% trichloroacetic acid[8]. This was followed by addition of 0.4 mL of 0.6% thiobarbituric acid solution. The tubes were subsequently incubated in boiling water for 20 minutes. The absorbance was measured at 532 nm in a spectrophotometer to determine the hydroxyl radical scavenging ability. [7] The Fe^{2+} chelating ability of both the plant extracts were determined using freshly prepared 500 mM $FeSO_4$ (150 mL) which was added to a reaction mixture containing 168 mL 0.1 M Tris–HCl (pH 7.4), 218 mL saline, and the plant extracts (0-0.32 mM). The reaction mixture was incubated for 5 minutes before the addition of 13 mL 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe (II) chelating ability was subsequently

calculated [9] were performed to determine the antioxidant potential of various extracts of *Morinda lucida* roots using already established methods. Statistical analysis was carried out using GraphPad Prism 8.0.2 software.

3. RESULTS AND DISCUSSION

The antioxidant properties of *Morinda lucida* root extracts (MRLD) were evaluated using assays, such as DPPH scavenging activity, FRAP, Fe^{2+} chelating ability and OH* radical scavenging ability. These assays provide insight into different mechanisms of antioxidant action, allowing for a comprehensive assessment of the extracts from *Morinda lucida*. For each of the Figures (1-3), the bars represent mean ± SEM (n=3).

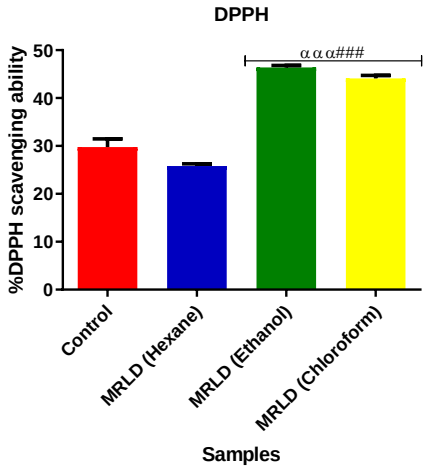


Figure 1: DPPH Scavenging ability of MRLD (Hexane, Ethanol and Chloroform) Extracts

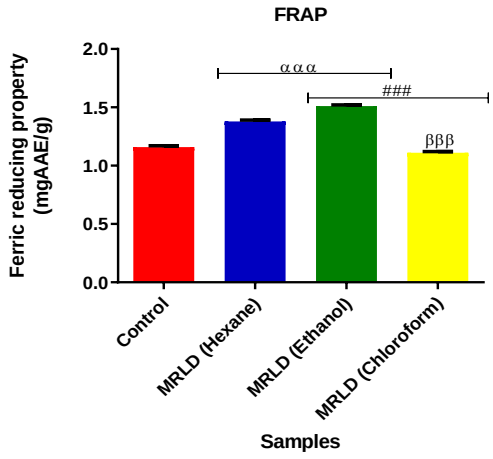


Figure 2: Ferric reducing antioxidant property of MRLD (Hexane, Ethanol and Chloroform) Extracts

3.1. DPPH Scavenging Activity

The DPPH assay results (Figure 1) indicate that the ethanol extract of *Morinda lucida* showed the best activity at 47.1 %, followed by the chloroform extract (45.6 %), while the hexane extract showed the lowest activity (26.7 %). The activities are statistically different at $^{\alpha\alpha\alpha}P < 0.001$, for kaempferol, $^{\#\#\#}P < 0.001$, for hexane as seen in Figure 1. Interestingly, both the ethanol and chloroform extracts demonstrated higher DPPH scavenging activity than the reference compound kaempferol (32.2 %). This suggests that polar and moderately polar solvents were more effective in extracting antioxidant compounds from *Morinda lucida* roots. The higher activity of the extracts compared to kaempferol may be due to the presence of a complex mixture of antioxidant compounds acting synergistically. [10]

3.2. Ferric Reducing Antioxidant Property (FRAP)

The FRAP assay as represented in milligram ascorbic acid per gram (mgAAE/g) (Figure 2) showed that the hexane, ethanol and chloroform extracts of *Morinda lucida* possessed reducing power, with the ethanol extract demonstrating the highest activity (1.53 mgAAE/g), followed by the hexane extract (1.40 mgAAE/g), and the chloroform extract (1.13 mgAAE/g). The ethanol and hexane extracts exhibited higher reducing power than the kaempferol control (1.18 mgAAE/g), indicating their potential as electron donors to neutralize free radicals. [7,9]

The result of the assay shows that they are statistically different at $^{\alpha\alpha\alpha}P < 0.001$, with Kaempferol (control),$^{\beta\beta\beta}P < 0.001$, for Ethanol, and $^{\#\#\#}P < 0.001$, for Hexane.

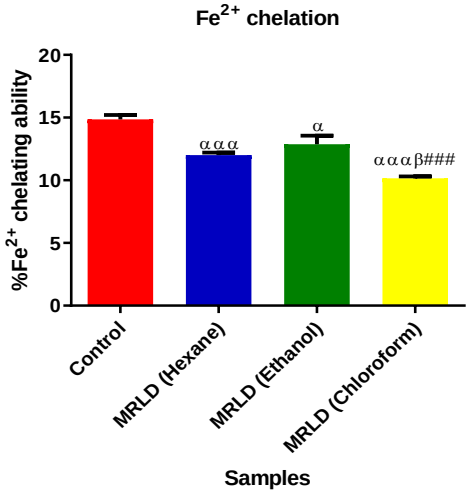


Figure 3: Fe^{2+} Chelating ability of MRLD (Hexane, Ethanol and Chloroform) Extracts

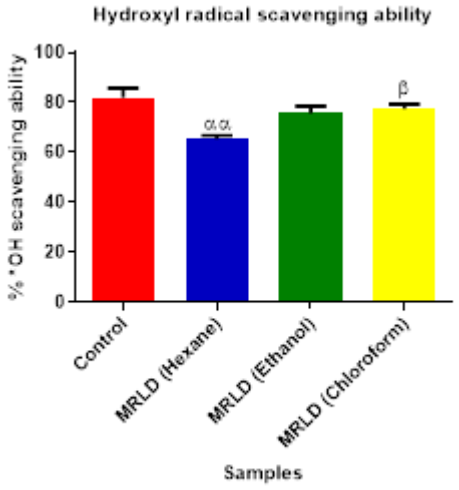


Figure 4: OH* Radical scavenging ability of MRLD (Hexane, Ethanol and Chloroform) Extracts

3.3. Fe^{2+} Chelating Ability

With respect to the chelating ability (Figure 3), the three extracts of *Morinda lucida* chelated iron but not as much as the kaempferol control, however, the ethanol extract (14.6 %) of *Morinda lucida* significantly chelated iron better than the hexane (12.6 %) and chloroform (10.5 %) extracts. The reference compound kaempferol showed slightly higher chelating ability (15.85 %). Values are statistically different at $^{\alpha\alpha\alpha}P < 0.001$, $^{\alpha}P < 0.05$ for kaempferol, $^{\beta}P < 0.05$ for Ethanol, $^{\#\#\#}P < 0.001$, for Hexane. Metal chelation is an important antioxidant mechanism, operating through metal-catalyzed reactions [11]. This result also revealed that the hexane, ethanol and chloroform extracts can scavenge hydroxyl radicals (OH*) from deoxyribose in Fenton reaction.

3.4. OH* Radical Scavenging Ability

The hydroxyl radical scavenging assay (Figure 4) demonstrated that all *Morinda lucida* extracts possessed considerable OH* radical scavenging ability. The ethanol extract had the best and most

consistent activity (70.7 %), followed by the chloroform extract (73.8 %), and the hexane extract (63.7 %). Notably, kaempferol exhibited higher OH* radical scavenging ability (81.0 %). Values are statistically different at $^{\alpha\alpha}P < 0.01$, for kaempferol, $^{\beta}P < 0.05$ for Ethanol. The hydroxyl radical is one of the most reactive and damaging species in biological systems, and the ability to scavenge these radicals is crucial for preventing oxidative stress.[12]

4. CONCLUSION

The results from these antioxidant assays consistently demonstrate that the ethanol extract of *Morinda lucida* roots possesses the strongest antioxidant activity across multiple mechanisms. This suggests that polar solvents like ethanol are most effective in extracting antioxidant compounds from *Morinda lucida* roots. The varying performance of the extracts in different assays highlights the importance of using multiple methods to evaluate antioxidant potential. The strong antioxidant activity of *Morinda lucida* root extracts, particularly the ethanol extract, suggests potential applications in pharmaceuticals and nutraceuticals. However, identifying and characterizing the specific antioxidant compounds present in these extracts and their safety in biological systems is essential.

REFERENCES

(1) Adekunle, O. D.; Adeleke, O. A.; Odugbemi, A. I.; Faboro, E. O.; Lajide, L. In vitro and in silico screening and identification of potential bioactive anthraquinones of *Morinda lucida* benth against pathogenic bacterial target proteins. *Disc App Sci*, **2024**, Vol 6 Issue 6, p 295.
(2) Adekunle, D. O.; Faboro, E. O.; Lajide, L. Molecular Docking and Pharmacokinetics Studies of Selected Anthraquinone Compounds with Possible Anti-Plasmodial Properties of *Morinda lucida*. *LIANBS,* **2024,** Vol 13 Issue 2, p 91 https://doi.org/10.33263/LIANBS132.091
(3) Adekunle, D.; Faboro, E.; Lajide, L. Identification and quantification of bioactive compounds indifferent extracts of *Morinda lucida* benth (rubiaceae) root using GC–MS analysis. *JNSPS* **2023**, p 1534-1534.
(4) Alsulami, F. J.; Shaheed, S. U. Role of Natural Antioxidants in Cancer. *Nutrition and Dietary Interventions in Cancer*, **2024,** p 95-117.
(5) Ramli, N. Z.; Yahaya, M. F.; Tooyama, I.; Damanhuri, H. A. A mechanistic evaluation of antioxidant nutraceuticals on their potential against age-associated neurodegenerative diseases. *Antioxidants*, **2020,** Vol 9 issue 10, p 1019.
(6) Yousaf, H. Evaluation and Comparison of the Antioxidant and Free Radical Scavenging Properties of Medicinal Plants by Using the DPPH Assay In-Vitro. *Biorxiv*, **2023**-01.
(7) Oboh, G.; Ogunsuyi, O. B.; Oyeleye, S. I.; Adefegha, S. A. Comparative Studies of the Effects of Five Indigenous Cowpea (*Vigna unguiculata*) Varieties on Enzymes Linked to Type 2 Diabetes and their Glycemic Indices. *TJNPR.* **2021** Vol 5 Issue 5 p 970-976
(8) Giriwono, P. E.; Iskandriati, D.; Tan, C. P.; Andarwulan, N. In-vitro anti-inflammatory activity, free radical (DPPH) scavenging, and ferric reducing ability (FRAP) of *Sargassum cristaefolium* lipid-soluble fraction and putative identification of bioactive compounds using UHPLC-ESI-ORBITRAP-MS/MS. *Food Research International*, **2020,** 137, 109702.
(9) Oboh, G.; Nwokocha, K. E.; Akinyemi, A. J.; Ademiluyi, A. O. Inhibitory effect of polyphenolic–rich extract from *Cola nitida* (Kolanut) seed on key enzyme linked to type 2 diabetes and Fe^{2+} induced lipid peroxidation in rat pancreas in vitro. *APJTB,* **2014,** 4, S405-S412.
(10) Lewandowska, U; Gorlach, S.; Owczarek, K.; Hrabec, E.; Szewczyk, K. Synergistic interactions between anticancer chemotherapeutics and phenolic compounds and anticancer synergy between polyphenols. *AHEM*, **2014** *68*, p 528-540.
(11) Santos-Sánchez, N. F.; Salas-Coronado, R.; Villanueva-Cañongo, C.; Hernández-Carlos, B. Antioxidant compounds and their antioxidant mechanism. *Antioxidants*, **2019,** *10*, 1-29.
(12) Lushchak, V. I. Free radicals, reactive oxygen species, oxidative stress and its classification. *Chemico-biological interactions*, **2014,** *224*, 164-175.