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Comparative Study of Antioxidant Activity of *Moringa Oleifera* Leaf and Seed Cultivated in Sokoto Metropolis

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Abstract

This study investigates and compares the antioxidant activity of *Moringa oleifera* leaves and seeds to identify which plant part exhibits stronger free radical scavenging potential. Antioxidant assays were conducted using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and the Total Phenolic Content (TPC) test. Methanolic extracts of both leaves and seeds were prepared via cold maceration and analyzed spectrophotometrically. The results revealed a concentration-dependent increase in antioxidant activity for both extracts. At 0.4 mg/mL, the leaf extract showed 82.67% inhibition, surpassing the seed extract (77.4%) and even the standard ascorbic acid (77.4%). The TPC analysis indicated slightly higher phenolic content in the leaf extract (30.4 mg GAE/100 g) compared to the seed extract (29.8 mg GAE/100 g). The enhanced antioxidant activity of the leaves is attributed to their higher phenolic and flavonoid composition. These findings demonstrate that *Moringa oleifera* leaves possess superior antioxidant potential relative to the seeds, supporting their application in nutraceutical, pharmaceutical, and food industries as natural antioxidant sources.

Keywords: Moringa oleifera, DPPH, antioxidant, cold maceration, phenolic content.

1.0 Introduction

Natural products are chemical compounds produced by living organisms, encompassing substances formed through biological processes [1]. They occur in two major categories: primary metabolites, which are essential for growth and reproduction, and secondary metabolites, which are not directly involved in these processes but provide adaptive advantages. In plants, secondary metabolites also known as phytochemicals are diverse organic compounds that can be extracted using different techniques, often involving organic solvents. Many of these metabolites have significant biological activities in humans, functioning as antibiotics, enzyme inhibitors, and growth promoters [2].

Medicinal plants play an essential role in maintaining human and community health due to their bioactive compounds that elicit specific physiological responses. These bioactive constituents include phenolic compounds, flavonoids, tannins, and alkaloids [3]. Plants synthesize these compounds as part of their defense

system against insects, pathogens, and environmental stresses [4]. Traditional medicinal herbs are widely used in rural and urban communities to treat various ailments [5]. Historical evidence indicates that many cultures have relied on medicinal plants for centuries because of their effectiveness, accessibility, and relatively low toxicity. It is estimated that over 75% of the global population depends on plant-derived remedies or their extracts for primary healthcare [6]. Depending on the plant part with the highest concentration of active compounds, remedies may involve using leaves, fruits, roots, or bark [7].

Phytoconstituents serve multiple ecological and physiological functions in plants, such as enhancing growth, activating defense mechanisms, and imparting distinctive flavors, colors, and aromas [8]. Some classes of secondary metabolites including terpenoids, steroids, and anthraquinones demonstrate notable biological activities such as anticancer, anti-inflammatory, anti-mutagenic, and antioxidant effects [9]. The process of phytochemical screening, involving extraction, isolation, and identification, is a critical step in drug discovery and development.

Oxidative stress occurs when there is an imbalance between the generation of reactive oxygen species (ROS) and the body's ability to neutralize them with antioxidant defenses (Sies, 2015). ROS, including free radicals such as superoxide anion ($O_2^{-}\bullet$) and hydroxyl radical (•OH), as well as non-radical species like hydrogen peroxide (H_2O_2), are natural byproducts of cellular metabolism, especially during mitochondrial respiration [10]. At low levels, ROS play roles in signaling and immunity, but excessive production causes damage to lipids, proteins, and DNA, thereby contributing to aging and chronic diseases such as cardiovascular disorders, cancer, and neurodegenerative conditions [11,12].

Antioxidants are molecules that inhibit oxidation by neutralizing ROS or reactive nitrogen species, thereby preventing cellular damage [13]. They function through mechanisms such as radical scavenging, metal ion chelation, and modulation of antioxidant enzyme activities [14]. Antioxidants are categorized into enzymatic (e.g., superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (e.g., vitamin C, vitamin E, carotenoids, polyphenols, flavonoids) groups [15].

Dietary antioxidants from fruits, vegetables, herbs, and spices significantly contribute to protection against oxidative stress [10]. Phytochemicals such as flavonoids, tannins, and phenolic acids exert antioxidative effects by donating electrons or hydrogen atoms to neutralize free radicals and by upregulating endogenous defense mechanisms [15,11]. Regular intake of antioxidant-rich foods reduces the risk of oxidative stress-related diseases [13].

Therefore, understanding the role of antioxidants in modulating oxidative stress is crucial for preventive and therapeutic medicine. Exploring plant-based antioxidants is particularly valuable, as they provide natural and accessible means of enhancing oxidative defense and promoting health.

1.1 Moringa oleifera

Moringa oleifera (Moringaceae), commonly called the "drumstick tree," "horseradish tree," "bean oil tree," "miracle tree," or "Mother's Best Friend" [16,17,18], is a fast-growing deciduous tree native to India, reaching 5–12 meters in height. It is now widely cultivated in tropical and subtropical regions of Africa, Arabia, Southeast Asia, South America, and the Caribbean [19]. The plant holds significant nutritional and medicinal value. The leaves are consumed fresh, cooked, or dried into powder for later use as food additives [20,21]. They are rich in vitamins, minerals, and proteins and are also used as animal feed [8,22]. The seeds contain alkaloids, flavonoids, saponins, and phenolic acids, which contribute to their characteristic taste [23,24]. Traditionally, preparations from seeds are used to manage diabetes, gastrointestinal issues, inflammation, and reproductive health disorders [25]. Both leaves and seeds demonstrate biological activities including anti-

tumor, anti-inflammatory, anti-ulcer, anti-atherosclerotic, and anticonvulsant effects [26,27,28]. These effects are attributed to their phytochemicals, particularly polyphenols.

Fig 1: Moringa Seeds (A) and Leaves (B)

Moringa oleifera is a slender, fast-growing tree, 5–12 m tall, with whitish-gray bark and feathery tripinnate leaves (30–60 cm long) [17]. The tree produces fragrant hermaphroditic flowers, which develop into triangular pods ("drumsticks") containing winged seeds [19]. Nutritionally, the leaves and seeds are rich in vitamins A, C, and E, as well as calcium, potassium, iron, and proteins [29,30].

Oxidative stress from free radicals contributes to diseases such as cancer, diabetes, cardiovascular disorders, and neurodegeneration. Antioxidants protect against oxidative damage, and medicinal plants are rich sources of these compounds. Moringa oleifera, the "miracle tree," is widely used for nutrition and medicine. While leaves have been extensively studied, seeds are less utilized despite evidence of bioactive compounds with antioxidant potential. There is limited comparative information on the antioxidant potential of Moringa oleifera leaves versus seeds. This knowledge gap necessitates research to determine which plant part provides stronger antioxidant benefits for nutritional, medicinal, and industrial applications. With increasing global interest in natural remedies and functional foods, the search for potent plant-based antioxidants has intensified. Moringa oleifera is accessible, affordable, and widely cultivated, making it a promising candidate for addressing oxidative stress-related health issues.

Although both leaves and seeds possess beneficial phytochemicals, most research focuses on leaves, leaving seeds underexplored. A comparative study will provide scientific evidence on their relative antioxidant potential. This knowledge will guide consumers, researchers, and industries in maximizing the nutritional and economic value of Moringa, particularly in food fortification, nutraceuticals, and pharmaceuticals.

1.2 Phytochemical Constituents of Moringa Leaf and Seed

Plants produce secondary metabolites called phytochemicals, which are frequently essential to their biological processes. Qualitative phytochemical screening of Moringa oleifera leaf and seed extracts showed the absence of tannins and phytosterols but the presence of alkaloids, saponins, phenolic compounds, and flavonoids [31]. The authors discovered that the total phenolic and flavonoid contents of the leaf extracts were significantly

higher than those of the seed extracts. This suggests that leaves are a more abundant source of antioxidant compounds.

Through phytochemical screening, [32] further verified that Moringa leaves contain tannins, flavonoids, saponins, and alkaloids. Their research revealed that these compounds work together to enhance Moringa leaves' antioxidant capacity. Flavonoids and phenolic compounds, in particular, are well-known for their ability to scavenge free radicals by donating hydrogen atoms or electrons, thereby neutralizing reactive species.

1.3 Mechanism of Antioxidant Action and Antioxidant Assays

In living systems, oxidative chain reactions that produce free radicals are disrupted by antioxidants. Although they are byproducts of regular metabolic processes, free radicals like reactive oxygen species (ROS) and reactive nitrogen species (RNS) can result in oxidative stress when they are produced in excess [39,40]. Atherosclerosis, diabetes, and neurodegenerative diseases are among the chronic diseases linked to oxidative stress, which also causes lipid peroxidation, DNA mutation, and protein oxidation [12].

Antioxidants derived from plants neutralize reactive species by donating electrons or hydrogen atoms, thereby reducing oxidative stress. By stopping radical chain reactions, this stabilizing effect turns dangerous radicals into innocuous molecules [41]. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is one of the most popular in vitro methods for evaluating antioxidant activity. When an antioxidant reduces the DPPH radical, its vivid violet hue turns yellow. The sample's ability to scavenge radicals is indicated by the drop in absorbance at 517 nm [42].

The reaction is represented as follows:

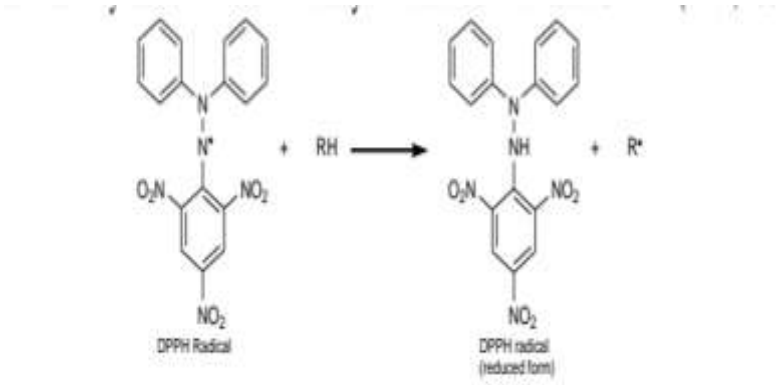

Fig. 2: The reaction of DPPH with antioxidant compound RH

The percentage inhibition of DPPH radicals is calculated using the equation:

$$\text{DPPH scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

In addition to the DPPH method, other assays are also employed to evaluate antioxidant potential. The Ferric Reducing Antioxidant Power (FRAP) assay measures the ability of antioxidants to reduce Fe^{3+} to Fe^{2+}, producing a blue-colored ferrous–tripyridyltriazine complex measured at 593 nm. Higher absorbance correlates with greater reducing power. Another method, the ABTS radical cation decolorization assay, utilizes the $ABTS^{+}$ radical, which reacts with antioxidants to form a colorless product, measured at 734 nm. The

Oxygen Radical Absorbance Capacity (ORAC) assay, in contrast, evaluates antioxidant protection against peroxyl radicals by monitoring fluorescence decay of a probe molecule [43].

These complementary assays provide a broader understanding of antioxidant mechanisms, allowing comparison of different antioxidant systems. However, among them, the DPPH assay remains the simplest and most widely applied in phytochemical studies of Moringa oleifera.

1.4 Comparative Antioxidant Activities of Moringa Leaf and Seed

Comparative studies have consistently demonstrated that Moringa oleifera leaves exhibit stronger antioxidant activity than seeds. [31] reported higher total phenolic (58.00–78.67 mg GAE/g) and flavonoid (31.73 mg QE/g) contents in leaves compared to seeds (17.67 mg GAE/g and 20.73 mg QE/g, respectively). These higher phytochemical levels corresponded with greater DPPH radical scavenging activity in leaves (IC_{50} = 35.42 µg/mL) than seeds (IC_{50} = 91.13 µg/mL).

This variation can be attributed to the functional roles of the plant parts: leaves act as primary photosynthetic organs and accumulate phenolic compounds for photoprotection, while seeds primarily store lipids and proteins for germination. Moreover, leaf extracts contain hydrophilic antioxidants like ascorbic acid and phenolic acids, while seeds contain more lipophilic antioxidants such as tocopherols and sterols [44].

Several studies corroborate these findings. For example, [45] observed that methanolic leaf extracts exhibited greater scavenging potential and higher ferric reducing power than seed extracts. Additionally, [46] demonstrated that Moringa leaf extracts possess higher inhibition of lipid peroxidation and hydrogen peroxide scavenging compared to seed oil extracts. These results suggest that while both plant parts contain antioxidants, the leaves are more potent due to their richer concentration of polyphenols.

Importantly, the combination of both leaves and seeds may produce synergistic antioxidant effects. The hydrophilic antioxidants from leaves and lipophilic components from seeds can collectively protect both aqueous and lipid environments within biological systems [37]. Such synergism is particularly relevant in nutraceutical formulations where whole-plant extracts are preferred over isolated compounds.

1.4 Factors Influencing Antioxidant Activity

The antioxidant capacity of *Moringa oleifera* is influenced by multiple intrinsic and extrinsic factors. The choice of extraction solvent significantly affects the yield and composition of antioxidants. Polar solvents like methanol, ethanol, and ethyl acetate generally extract higher amounts of phenolic compounds, whereas nonpolar solvents such as hexane are more effective for isolating lipid-soluble antioxidants [47,48]. Secondary metabolite synthesis is also influenced by environmental factors such as temperature, altitude, and soil composition. Because highland plants are exposed to more UV light and colder temperatures, which cause stress-related biosynthesis of flavonoids and phenolics, they tend to accumulate more antioxidants [49]. According to [32], lowland Moringa leaves had less antioxidant activity than their highland counterparts, most likely as a result of lower vitamin C levels.

Antioxidant concentration is also influenced by the plant's maturity stage. Because of their active metabolism during growth, young leaves have been found to have higher levels of antioxidant activity and total phenolic content than older leaves [50]. Storage conditions, drying temperature, and postharvest handling are also important factors. Heat-sensitive antioxidants like ascorbic acid and carotenoids can degrade when exposed to high temperatures and stored for an extended period of time [44]. Therefore, to guarantee consistent

antioxidant profiles in products derived from moringa, standardization of cultivation and extraction parameters is crucial. Comprehending these variables is essential for industrial applications as well as research comparability.

3.0. Materials and Method

3.1. Materials

All chemicals and reagents used in this study were of analytical grade and were used without further purification. Methanol (98%), ethanol (97%), sodium carbonate (Na_2CO_3), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, gallic acid, ascorbic acid, and distilled water were used for extraction and antioxidant analyses. The laboratory equipment employed included a Pyrex glass conical flask (Pyrex, England), glass pipettes, glass test tubes (Pyrex, England), an incubator, a rotary evaporator (SRC5, Cole-Parmer TD), a DZ-6020 dry air oven (China), a PC-440 analytical balance (Mettler, Switzerland), and a UV–Visible spectrophotometer (Kyoto, Japan).

3.2 Preparation of Reagents

A 0.1 mM DPPH solution was prepared by dissolving 0.00394 g of DPPH in 100 mL of methanol using a volumetric flask and protecting the solution from light until use. A 10% (v/v) Folin–Ciocalteu reagent was prepared by diluting 10 mL of the commercial reagent to a final volume of 100 mL with distilled water. A 7.5% (w/v) sodium carbonate solution was prepared by dissolving 0.75 g of sodium carbonate in 10 mL of distilled water with continuous stirring until complete dissolution.

3.3 Collection and Preparation of Plant Materials

Fresh *Moringa oleifera* leaves were collected from the Fishery Garden, while mature *Moringa oleifera* seeds were obtained from a local market within Sokoto Metropolis, Sokoto State, Nigeria. The collected leaves were thoroughly washed with clean water to remove adhering dirt and other foreign materials and subsequently air-dried at room temperature until a constant weight was achieved. The dried leaves were pulverized into a fine powder using a clean electric blender. The seeds were manually dehulled, air-dried under ambient laboratory conditions, and similarly ground into a fine powder. The powdered leaf and seed samples were separately stored in clean, airtight containers in a cool and dry environment until further analysis.

3.4 Extraction of *Moringa oleifera* Leaves and Seeds

Methanolic extracts of *Moringa oleifera* leaves and seeds were prepared separately using the cold maceration technique. Briefly, 10 g of each powdered sample was accurately weighed into separate clean conical flasks, and 100 mL of 80% methanol was added. The mixtures were allowed to macerate for 48–72 h at room temperature with intermittent shaking to facilitate efficient extraction of bioactive constituents. Following extraction, the mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at a temperature not exceeding 40°C to remove the extraction solvent. The concentrated extracts were transferred into airtight containers and stored at 4°C until required for antioxidant analyses.

3.5 Comparative Antioxidant Analysis of *Moringa oleifera* Leaf and Seed Extracts

The antioxidant properties of the methanolic extracts obtained from *Moringa oleifera* leaves and seeds were comparatively evaluated using the DPPH free radical scavenging assay and the Folin–Ciocalteu total phenolic

content (TPC) assay. These methods were selected because they provide complementary information on the antioxidant potential of plant extracts by assessing both free radical scavenging activity and total phenolic constituents.

3.5 DPPH Radical Scavenging Assay

The free radical scavenging activity of the leaf and seed extracts was determined according to the DPPH method described by [51] with slight modifications. Briefly, 1.0 mL of freshly prepared 0.1 mM DPPH solution was mixed with 1.0 mL of each extract prepared at concentrations ranging from 0.2 to 0.4 mg/mL. The reaction mixtures were vortexed thoroughly and incubated in the dark at room temperature for 30 min to ensure complete interaction between the antioxidants present in the extracts and the DPPH radicals. Thereafter, the absorbance of each reaction mixture was measured at 517 nm using a UV–Visible spectrophotometer, with methanol serving as the blank. Ascorbic acid was used as the positive control. The percentage radical scavenging activity was calculated according to the following equation:

$$\text{DPPH Scavenging Activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where $A_{\text{control}}$is the absorbance of the DPPH solution without the extract and $A_{\text{sample}}$is the absorbance of the DPPH solution containing the extract.

3.6 Determination of Total Phenolic Content

The total phenolic content of the methanolic leaf and seed extracts was determined using the Folin–Ciocalteu colorimetric method as previously described by [52] with slight modifications. Briefly, 0.5 mL of each extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent, thoroughly mixed, and allowed to stand in the dark for 5 min at room temperature. Subsequently, 2.0 mL of 7.5% sodium carbonate solution was added, and the resulting mixture was incubated for an additional 30 min at room temperature to allow colour development. The absorbance was then measured at 760 nm using a UV–Visible spectrophotometer, with methanol used as the blank. The phenolic concentration of each extract was determined from a gallic acid calibration curve (100–500 µg/mL) using the regression equation $Y = 0.002x + 0.0511$ ($R^2 = 0.966$), and the results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). The total phenolic content was calculated using the equation:

$$\text{TPC} = \frac{C \times V}{m}$$

where C is the concentration of phenolic compounds obtained from the gallic acid calibration curve (mg/mL), V is the volume of extract used (mL), and m is the mass of the extract (g).

4.0 Results and Discussion

4.1 Results

The antioxidant activity of Moringa oleifera leaf and seed extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The assay determines the ability of antioxidants to donate hydrogen atoms or electrons to neutralize free radicals, measured by a decrease in absorbance at (517 nm.)

4.1.1 DPPH Radical Scavenging Activity

The results presented in Tables below show that both Moringa leaf and seed extracts exhibited marked antioxidant activities, which increased with extract concentration, demonstrating a dose-dependent relationship.

Table 1: Antioxidant Activity of Moringa Seed Extract

Concentration	Absorbance of sample at 517nm	% Inhibition of sample	Absorbance of Ascorbic Acid at 517nm	% Inhibition of Ascorbic Acid
Control	1.470	-	1.470	-
0.2	0.374	74.5 ± 0.87	0.415	71.76
0.3	0.341	76.7 ± 0.68	0.351	76.12
0.4	0.332	77.4 ± 2.88	0.336	77.14

Table 2: Antioxidant Activity of Moringa Leaf Extract

Concentration	Absorbance of sample at 517nm	% Inhibition of sample	Absorbance of Ascorbic Acid at 517nm	% Inhibition of Ascorbic Acid
Control	1.470	-	1.470	-
0.2	0.391	73.4 ± 0.21	0.415	71.76
0.3	0.317	78.4 ± 0.20	0.351	76.12
0.4	0.255	82.67 ± 1.09	0.336	77.40

At a concentration of (0.2 mg/ml), both extracts demonstrated substantial radical scavenging activity, with percentage inhibitions of (74.5%) for the seed and (73.4%) for the leaf. As the concentration increased to 0.4 mg/ml, the inhibitory activity rose to (77.4%) for the seed and (82.67%) for the leaf, indicating a clear concentration-dependent enhancement in antioxidant potential.

Phenolic compounds are key contributors to antioxidant capacity due to their redox properties. The total phenolic content (TPC) of the extracts was determined and is summarized in Table 3.

4.1.2 Total Phenolic Content (TPC)

Table 3: Total Phenolic Content (TPC) of Moringa Leaf and Seed

Sample	Concentration (mg/ml)	TPC (mg GAE/100g)
Moringa Leaf	304.45	30.4
Moringa Seed	298.45	29.8

The Moringa leaf extract exhibited slightly higher phenolic content (30.4 mg GAE/100g) compared with the seed extract (29.8 mg GAE/100g). This difference supports the observation that higher phenolic concentration correlates with greater antioxidant activity.

4.2 Discussions

The findings of this study demonstrate that both Moringa oleifera leaf and seed extracts possess strong antioxidant activity, increasing with concentration in a dose-dependent manner. This pattern aligns with the general behavior of polyphenol-rich plant extracts, where a greater concentration of active compounds leads to enhanced radical scavenging ability.

The higher DPPH scavenging activity observed in Moringa leaf extract compared to the seed extract may be attributed to the presence of higher levels of polyphenolic compounds and other phytochemicals such as flavonoids, phenolic acids, and vitamins [53,7]. These bioactive compounds act as reducing agents and hydrogen donors, stabilizing free radicals and preventing oxidative damage.

At (0.4 mg/ml), Moringa leaf extract exhibited (82.67%) inhibition, surpassing both the seed extract (77.4%) and the standard ascorbic acid (77.4%). This suggests that Moringa leaf extract could serve as an efficient natural antioxidant source comparable or superior to synthetic antioxidants. The results were statistically validated through one-way ANOVA, revealing significant differences ($p < 0.001$) among concentrations. Furthermore, Pearson correlation analysis yielded a strong positive correlation ($r = +0.98$, $p < 0.01$) between concentration and inhibition percentage, confirming a concentration-dependent antioxidant effect.

These findings are consistent with the works of [53], who reported high antioxidant activity in Moringa leaves, and [54], who observed significant antioxidant and antimicrobial properties in Moringa seed extracts. Such consistency reinforces the robustness of the present study's outcomes.

From a biochemical perspective, the superior performance of Moringa leaf extract may be linked to its richer diversity of flavonoids (e.g., quercetin, kaempferol), phenolic acids (e.g., chlorogenic acid), and ascorbic acid, all of which act synergistically to enhance antioxidant defense mechanisms. These compounds neutralize reactive oxygen species (ROS) through mechanisms such as electron transfer, hydrogen atom donation, and metal ion chelation [40].

5. Conclusion

The study clearly demonstrates that Moringa leaf and seed extracts possess significant antioxidant activity, with leaves showing superior effects likely due to higher phenolic content. Their activity rivals or exceeds that of ascorbic acid, highlighting the potential of Moringa as a natural antioxidant source. These findings have promising applications in health, nutrition, and industry, warranting further detailed phytochemical and biological studies.

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