In-vitro Antioxidant properties of Moringa *oleifera* Leaf Extract in Management of Oxidative Stress.

BY

Izibili Naomi Oseotue

Bms2101419

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CERTIFICATION

We, the undersigned, hereby certify that Izibili Naomi Oseotue (Bms2101419) carried out this work in the Department of Medical Biochemistry, University of Benin, Benin City, and we approve the same as adequate in scope and quality for the reward of Bachelor of Science Degree (B.Sc.) in Medical Biochemistry.

Signed:

_____ _____

Dr L.O Agbontaen (Date)

(Project Supervisor)

_____ _____

Dr B.N. AGUEBOR-OGIE (Date)

(Head of Department)

_____ _____

(Date)

(External Examiner)

DEDICATION

I dedicate to God Almighty and my wonderful parents. Mr and Mrs Izibili.

ACKNOWLEDGEMENT

I would love to acknowledge the head of the department, Dr B.N. AGUEBOR-OGIE and all the amazing lecturers of the Department of Medical Biochemistry.

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Thank you all, God bless you!

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ABSTRACT

M*oringa oleifera* is widely valued for its medicinal and nutritional importance, particularly its rich content of bioactive compounds with antioxidant properties. This study aimed to evaluate the in vitro antioxidant activity of M*oringa oleifera* leaf extract and assess its potential role in the management of oxidative stress. Fresh Moringa leaves were collected, washed, weighed, homogenised, and extracted using standard solvent-extraction procedures to obtain the crude leaf extract used for analysis.

The antioxidant capacity of the extract was examined using three established assays: the Nitric Oxide (NO) scavenging assay, the Ferric Reducing Antioxidant Power (FRAP) assay, and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Ascorbic acid served as the reference standard. Results showed that the Moringa extract exhibited considerable antioxidant activity across all assays, with NO inhibition ranging from 36.34–57.42%, FRAP reducing power from 46.68–58.58%, and DPPH radical scavenging from 57.44–84.21%. Although these values were lower than those of pure ascorbic acid, the extract still demonstrated strong free radical–neutralizing and electron-donating abilities.

The significant in-vitro activity observed in this study provides scientific justification for its traditional use as a natural antioxidant and suggests that M*oringa oleifera* is a promising candidate for future in-vivo studies aimed at exploring its therapeutic potential in oxidative stress–related conditions.

CHAPTER 1

1.0 INTRODUCTION

1.1 Background of the Study

Oxidative stress has been defined as a disturbance in the balance between the production of reactive oxygen species (ROS) or free radicals and antioxidant defences. Many metabolic reactions depend on oxygen, a primary oxidant in metabolic reactions designed to obtain energy from the oxidation of a variety of organic molecules, which is also used as substrate by numerous enzymes, each with differing substrate affinities for oxygen and involved in a wide range of metabolic processes such as the metabolism of amines, prostaglandins, purines, steroids, and amino acids.

ROS are generated as a by-product of normal aerobic metabolism, but their level increases under stress, which proves to be a basic health hazard. These ROS include superoxide (O2-), hydroxyl radical (HO.), and non-radical molecules like hydrogen peroxide (H2O2). Nitric oxide (NO) is another abundant reactive radical that acts as an important oxidative biological signal in a large variety of diverse physiological processes, including smooth muscle relaxation, neurotransmission, and immune regulation

Antioxidants are compounds that inhibit or delay oxidation processes by scavenging free radicals or chelating transition metal ions that catalyze oxidative reactions (Phaniendra *et al.*, 2015). Although the human body possesses intrinsic antioxidant systems such as

catalase, superoxide dismutase, and glutathione peroxidase, these may not be sufficient under conditions of excessive oxidative stress. Therefore, there is a growing interest in dietary and plant-derived antioxidants as potential therapeutic agents.

M*oringa oleifera*, commonly known as the "drumstick tree" or "miracle tree," is a plant of the family Moringaceae. It is native to the Indian subcontinent but is now widely cultivated across Africa and Asia for its nutritional and medicinal values (Anwar *et al.,* 2007). The leaves of M*oringa oleifera* are rich in phenolic compounds, flavonoids, tannins, vitamins (A, C, and E), and minerals, which contribute to its high antioxidant potential (Sreelatha & Padma, 2009).

Several studies have reported the strong in-vitro antioxidant activity of M*oringa oleifera* leaf extract using different assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and nitric oxide scavenging assays (Nouman *et al.*, 2016; Moyo *et al.,* 2012). These bioactive components act synergistically to neutralize free radicals, reduce oxidative stress, and protect biological systems against oxidative damage.

Thus, the evaluation of the in-vitro antioxidant properties of M*oringa oleifera* leaf extract is essential for scientifically validating its traditional use and exploring its potential role in the management of oxidative stress–related diseases.

1.2 Statement of the Problem

Oxidative stress contributes to the onset and progression of several metabolic and degenerative diseases, such as diabetes mellitus, atherosclerosis, and cancer (Valko *et al*., 2007). The conventional synthetic antioxidants used in preventing oxidative damage, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been linked with toxic and carcinogenic side effects (Kahl & Kappus, 1993).

This has led to a shift toward the use of natural antioxidants, especially from plant sources, which are considered safer and more effective. Despite the traditional medicinal use of M*oringa oleifera*, there remains a need for more scientific evaluation of its antioxidant potential under in vitro conditions. This research, therefore, seeks to assess the in vitro antioxidant properties of M*oringa oleifera* leaf extract to establish its role in managing oxidative stress.

1.3 Aim of the Study

This study aims to evaluate the in vitro antioxidant properties of M*oringa oleifera* leaf extract in the management of oxidative stress.

1.4 Objectives of the Study

The specific objectives of this study are to:

1. Identify the phytochemical constituents of M*oringa oleifera* leaf extract.
2. Evaluate the in vitro antioxidant activities of M*oringa oleifera* leaf extract using standard assays such as DPPH radical scavenging activity.
3. Compare the antioxidant potential of M*oringa oleifera* with standard antioxidants.
4. Assess the possible role of M*oringa oleifera* in the management of oxidative stress.

1.5 Significance of the Study

This study will provide scientific evidence supporting the traditional use of *Moringa oleifera* as a natural antioxidant source. The findings will:

1. Contribute to the understanding of the antioxidant mechanisms of M*oringa oleifera;*
2. Promote the use of natural antioxidants as safer alternatives to synthetic compounds.
3. Offer a biochemical basis for the development of plant-based therapeutic agents;
4. Serve as reference material for future research on antioxidant-rich medicinal plants.

1.6 Scope of the Study

This research focuses on the in vitro antioxidant properties of M*oringa oleifera* leaf extract. The study is limited to phytochemical screening and antioxidant assays such as DPPH radical scavenging and ferric reducing antioxidant power (FRAP). It excludes in vivo studies, clinical trials, and analysis of other plant parts (seeds, roots, or bark).

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Overview of M*oringa oleifera*

Moringa (M*oringa oleifera* Lam.) is a multipurpose tropical tree. It is mainly used for food and has numerous industrial, medicinal and agricultural uses, including animal feeding. Nutritious, fast-growing and drought-tolerant, this traditional plant was rediscovered in the 1990s, and its cultivation has since become increasingly popular in Asia and Africa, where it is among the most economically valuable crops. It has been dubbed the "miracle tree" or "tree of life" by the media (FAO, 2014; Radovich, 2009; Orwa *et al*., 2009; Bosch, 2004). Native to the northwestern region of India, M*oringa oleifera* is now cultivated widely across Africa, Asia, and South America due to its adaptability to diverse climatic conditions (Fahey, 2005). Nearly every part of the tree, leaves, seeds, pods, bark, and roots, possesses beneficial properties, making it one of the most versatile plants known.

Fig 2.1: M*oringa oleifera* leaves. ECHO Research Notes 1.

2.1.1. Botanical Description of M*oringa oleifera*

M*oringa oleifera* is a fast-growing, deciduous tree that can reach heights of 10–12 meters. It thrives in semi-arid, tropical, and subtropical climates and is drought-resistant once established (Anwar *et al.*, 2007).

Key botanical features include:

Leaves: The leaves are tripinnate, pale green, and compound, consisting of small, oval leaflets. They are the most nutritionally valuable part of the plant, containing high levels of vitamins, minerals, and antioxidants (Moyo *et al*., 2012).

Flowers: The plant produces small, fragrant, white or cream-colored flowers arranged in clusters. The flowers are rich in nectar and are a good source of pollen for bees (Fahey, 2005).

Fruits: The fruit is a long, slender, triangular pod known as a "drumstick," containing winged seeds.

Seeds: The seeds are round with three papery wings and yield high-quality oil, commonly referred to as "ben oil," used in cosmetics and cooking.

Roots and Bark: The roots have a pungent taste similar to horseradish and possess antimicrobial and anti-inflammatory properties (Leone *et al*., 2015).

2.1.2 Historical and Cultural Relevance of M*oringa oleifera*

The use of M*oringa oleifera* dates back to ancient civilizations. Historical records from India and Egypt indicate its use as early as 2000 B.C. for medicinal purposes and water purification (Fahey, 2005). In Ayurvedic medicine, Moringa is believed to prevent over 300 diseases. Ancient Egyptians used Moringa oil for skin protection against the desert climate, while the Greeks and Romans employed it as a perfume and ointment base (Leone *et al*., 2015).

In Africa, Moringa is traditionally used as food and medicine. The Hausa call it "Zogale", the Yoruba "Ewe Igbale", and the Igbo "Okwe Oyibo". It is commonly cultivated around homes and used in soups, teas, and herbal remedies due to its nutrient-dense leaves (Gopalakrishnan *et al*., 2016).

2.13: Morphological Characteristics of M*oringa oleifera*

Feature	Description
Growth habit	Fast-growing, deciduous tree, 10–12 m tall
Trunk	Soft, whitish-grey, up to 45 cm in diameter
Leaves	Alternate, tripinnate, with small oval leaflets

Flowers	Creamy-white, fragrant, bisexual, zygomorphic
Pods	Long, pendulous, triangular, green turning brown when mature
Seeds	Globular, brownish-black with papery wings
Root	Thick taproot, deep-penetrating, pungent
Life span	Perennial
Habitat	Thrives in well-drained loamy or sandy soils; tolerant to drought
Reproduction	By seeds and stem cuttings

2.1.4: Taxonomy and Classification of M*oringa oleifera*

M*oringa oleifera* is the most widely cultivated species of the genus Moringa, which comprises about 13 known species. The scientific classification is as follows:

1. Kingdom: Plantae
2. Division (Phylum): Magnoliophyta:
3. Class: Magnoliopsida:
4. Order: Brassicales:
5. Family: Moringaceae:
6. Genus: Moringa:

7. Species: M*oringa oleifera* Lam.:

2.2 Oxidative stress

Oxidative stress refers to a physiological condition that arises when there is an imbalance between the production of reactive oxygen species (ROS) (also known as free radicals) and the ability of the body's antioxidant defense systems to neutralize or detoxify them. This imbalance leads to cellular and molecular damage affecting lipids, proteins, and DNA.

ROS are produced as normal by-products of cellular metabolism (mitochondrial electron transport, NADPH oxidases, xanthine oxidase, peroxisomal reactions) and by immune cells (respiratory burst). When ROS/RNS levels exceed the buffering capacity of antioxidant systems, they react with lipids, proteins and DNA causing lipid peroxidation, protein oxidation, enzyme inactivation, DNA strand breaks and mutagenesis — processes that drive cell dysfunction, apoptosis or necrosis and chronic inflammatory signalling (NF-κB, MAPK).

2.2.1 Mechanism of Oxidative Stress

1. Generation of ROS: ROS are produced naturally during mitochondrial respiration, enzymatic reactions, and immune defense mechanisms. However, environmental factors such as pollution, UV radiation, smoking, or toxins can increase their production.

2. Inadequate Antioxidant Defense: The body uses enzymatic antioxidants (like superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic antioxidants (like vitamin C, vitamin E, flavonoids, and polyphenols) to neutralize ROS. When these are depleted or insufficient, oxidative stress develops.

3. Damage to Biomolecules:

- Lipid peroxidation: ROS attack polyunsaturated fatty acids in cell membranes, impairing membrane integrity.
- Protein oxidation: Alters enzyme function and structural proteins.
- DNA damage: Leads to mutations, ageing, and carcinogenesis.

2.2.2 Consequences of Oxidative Stress

Oxidative stress has been implicated in a wide range of chronic and degenerative diseases, including:

1. Cardiovascular diseases:(atherosclerosis, hypertension)
2. Diabetes mellitus
3. Neurodegenerative diseases: (Alzheimer's, Parkinson's)
4. Cancer
5. Chronic inflammation
6. Liver and kidney dysfunction
7. Ageing-related disorders

2.2.3 Management of oxidative stress.

Oxidative stress can be managed by:

1. Increasing dietary intake of antioxidants (found in fruits, vegetables, and herbal plants like M*oringa oleifera)*.

2. Reducing exposure to oxidative stress-inducing factors (e.g., smoking, alcohol, radiation).

3. Supporting enzymatic antioxidants through nutrient-rich diets that enhance glutathione and related enzymes.

2.3 Antioxidants

Antioxidants are substances capable of preventing or slowing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent, leading to the generation of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). These free radicals can damage cell membranes, proteins, lipids, and DNA, contributing to cellular dysfunction and chronic diseases (Lobo et al., 2010; Halliwell & Gutteridge, 2015).

Antioxidants act by neutralizing these free radicals, thus protecting biological molecules and maintaining redox homeostasis in cells.

They can be enzymatic or non-enzymatic, natural (from plants or organisms) or synthetic (manufactured chemically for therapeutic use).

2.3.1 Assays for determining antioxidant properties:

1. DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay.

The DPPH radical (a stable nitrogen-centred free radical with violet colour) accepts an electron or hydrogen from an antioxidant, producing a colour change (purple → yellow/colourless). PMC+1

A known concentration of DPPH in solution (often methanol) is mixed with the antioxidant/sample. After an incubation period (often 30 min or more in the dark), the decrease in absorbance (commonly at ~517 nm) is recorded. Results can be expressed as % inhibition or IC_{50} (concentration needed to reduce DPPH by 50%). This assay is simple, fast, inexpensive, and widely used, so much comparative data exists.

2. ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid))

It is a Radical Cation Decolourization Assay. The ABTS radical cation ($ABTS^{+}$•) is generated (typically via oxidation, e.g., potassium persulfate) and has a blue-green colour; antioxidants reduce $ABTS^{+}$• to ABTS, causing decolourisation (monitored at ~734 nm).

After generating $ABTS^{+}$•, mix with the sample/extract, and measure the reduction of absorbance after a defined time. Often, results are expressed relative to a standard antioxidant, e.g., Trolox equivalents (TEAC = Trolox-Equivalent Antioxidant Capacity).

This assay is applicable to both hydrophilic and lipophilic antioxidants, with flexible assay conditions.

3. FRAP (Ferric Reducing Antioxidant Power) Assay: It measures the ability of antioxidants to reduce Fe^{3+}-TPTZ (2,4,6-tripyridyl-s-triazine) complex to the ferrous form (Fe^{2+}), which forms a blue complex measured at ~593 nm.

FRAP reagent is prepared using (acetate buffer + TPTZ + $FeCl_3$). Add sample, incubate (often ~10 min at 37 °C), measure increase in absorbance. Express results as Fe^{2+} equivalents or as Trolox equivalents.

This assay gives a straightforward measure of "reducing capacity" (often considered a surrogate for antioxidant capacity). It only measures reducing ability under acidic conditions; does not account for radical scavenging kinetics; may not reflect lipid-phase or cellular antioxidant capacity.

4. Metal Chelation Assays (e.g., Fe^{2+} Chelating, Ferrozine Method)

Principle: Since metal ions such as Fe^{2+} catalyse ROS production via Fenton/Haber-Weiss reactions, antioxidants that chelate these ions reduce pro-oxidant activity. The method often uses ferrozine, which forms a magenta complex with Fe^{2+}; chelators reduce complex formation, reducing absorbance (commonly ~562 nm).

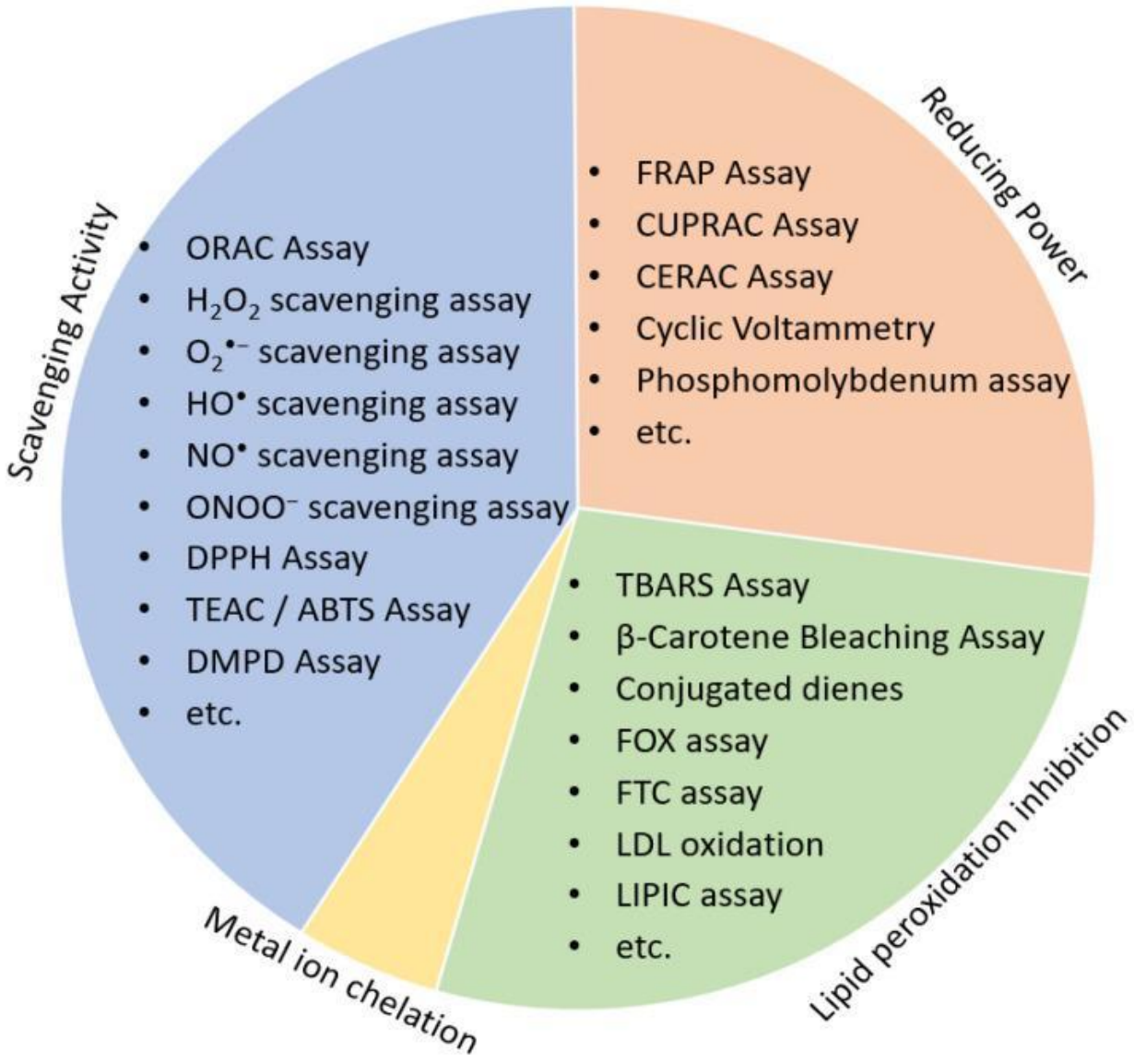

Figure 2.2: Major categories of antioxidant assays based on their mechanism of action, including scavenging activity, reducing power, metal ion chelation, and lipid peroxidation inhibition (Yin & Xu, 2022).

2.3.2 Sources of Antioxidants

Antioxidants are compounds that prevent or slow oxidative damage by neutralizing free radicals in the body. They can be obtained from endogenous (internal) and exogenous (dietary or natural) sources.

1. Endogenous (Internal Sources): These are antioxidants that the body naturally synthesises to maintain redox balance and protect against oxidative stress. They are mostly enzymatic and non-enzymatic molecules.

a. Enzymatic Antioxidants: These enzymes catalyze reactions that convert reactive oxygen species (ROS) into less harmful compounds.For example:

Catalase (CAT) breaks down hydrogen peroxide into water (H_2O) and oxygen (O_2).

b. Non-Enzymatic Endogenous Antioxidants: These are low-molecular-weight compounds that scavenge free radicals directly.

2. Exogenous (Dietary or Natural) Sources: These are antioxidants obtained from diet, plants, and supplements. They include vitamins, minerals, phenolic compounds, flavonoids, and carotenoids.

a. Vitamins

Vitamin C (Ascorbic Acid): A water-soluble antioxidant found in citrus fruits, tomatoes, and green leafy vegetables. It donates electrons to neutralize ROS.

Vitamin E (Tocopherols and Tocotrienols): A fat-soluble antioxidant found in nuts, seeds, vegetable oils, and whole grains that protects cell membranes from lipid peroxidation.

Vitamin A (Retinoids and Carotenoids): Found in carrots, spinach, and sweet potatoes; protects tissues and boosts immune function.

b. Minerals

Certain trace elements act as cofactors for antioxidant enzymes:

Selenium: Required for glutathione peroxidase activity (found in Brazil nuts, fish, and grains).

Zinc and Copper: Essential for superoxide dismutase function (found in beans, shellfish, and whole grains).

Manganese: Also a cofactor for SOD in mitochondria.

c. Polyphenols and Flavonoids: These are plant-derived secondary metabolites with strong antioxidant potential:

Phenolic acids (e.g., caffeic acid, ferulic acid) — found in coffee, fruits, and grains.

Flavonoids (e.g., quercetin, catechins, anthocyanins) — abundant in berries, tea, onions, and apples.

Tannins and lignans — found in nuts, seeds, and legumes.

d. Carotenoids: These are pigments in fruits and vegetables with antioxidant properties: β-carotene, lycopene, lutein, and zeaxanthin protect against lipid peroxidation and oxidative damage in the eyes and skin.

Sources: carrots, tomatoes, spinach, and red peppers.

e. Other Natural Sources

Plant extracts: M*oringa oleifera*, green tea, turmeric (curcumin), and garlic are rich in phytochemicals with antioxidant effects.

Beverages: Green tea, coffee, and red wine contain polyphenols like catechins and resveratrol.

Whole grains and legumes: Provide phenolics and vitamin E.

Spices and herbs: Clove, cinnamon, rosemary, and ginger contain potent antioxidant compounds.

2.3.3 Diet-Related Illnesses Associated with Oxidative Stress

Deficiency of dietary antioxidants or excess production of ROS leads to oxidative stress, which plays a role in the development of many diet-related and chronic illnesses:

Cardiovascular Diseases: Oxidation of low-density lipoprotein (LDL) cholesterol leads to atherosclerosis.

Diabetes Mellitus: Chronic hyperglycemia enhances ROS production, causing insulin resistance and β-cell dysfunction.

Cancer: Oxidative DNA damage promotes mutations and carcinogenesis.

Obesity and Metabolic Syndrome: Fat accumulation increases ROS production, leading to inflammation.

2.3.4 Health Benefits of Antioxidants

Antioxidants contribute to overall health by supporting cellular integrity and modulating oxidative and inflammatory pathways.

Major health benefits include:

1. Protection Against Chronic Diseases.
2. Anti-Aging Effects.
3. Anti-Inflammatory Effects.
4. Immune System Support.
5. Liver Protection.
6. Metabolic Regulation.
7. Neuroprotection.

2.4 How the antioxidant properties of M*oringa oleifera* can manage oxidative stress

M*oringa oleifera* extracts possess anti-oxidative and anti-inflammatory properties, which have been attributed to its polyphenols, flavonoids (particularly quercetin and phenolic acids. The alkaloids, flavonoids, saponins, triterpenoids/steroids and tannins in M. oleifera extract are powerful antioxidants that act to prevent new free radicals and chain reactions and protect the cells from oxidative damage.

The Transition metals (e.g., Fe^{2+}, Cu^{2+}) catalyze the formation of reactive oxygen species via Fenton reactions.

M*oringa oleifera* leaf polyphenols and tannins bind these metal ions, preventing them from initiating oxidative reactions.

Example: By chelating Fe^{2+}, Moringa extracts reduce hydroxyl radical generation from hydrogen peroxide, thus limiting cellular damage.

M*oringa oleifera* not only provides external antioxidants but also stimulates the body's natural antioxidant defense systems. Studies show that Moringa supplementation can stimulate enzymes to detoxify ROS, maintain redox balance, and protect against oxidative injury in tissues such as the liver, kidneys, and brain.

CHAPTER 3

3.0 MATERIALS AND METHOD

3.1 Experimental design

The experiment was designed to analyze the antioxidant properties of M*oringa oleifera* leaves by assessing its efficacy using the various assays, like DPPH and FRAP, for antioxidant extraction. This research was carried out to ensure that the sample was properly prepared and filtered to attain the highest quality extracts. The experimental procedures were done with a systematic approach, performing the analysis at regular intervals to ensure the reliability of the results.

3.2 Materials

Reagent

Distilled water

Methanol

2,2-diphenyl-1-picrylhydrazyl (DPPH)

Nitric oxide

Sodium nitroprusside

Sulfanilic acid

Glacial acetic acid

FeSO4

Ascorbic acid

Ferric chloride

Tripyridyl Triazine (TPTZ)

Molybdate

Sodium nitroprusside

Glacial acetic acid

Nathylethylene diamine dihydrochloride

3.3. APPARATUS

The following apparatus was used:

1. Test Tubes

2. Test Tube Racks

3. Mortar and Pestle

4. Beakers (50 mL, 100 mL, 200 mL)

5. Pipette:

6. Micro Pipette

7. Measuring Cylinder

8. Masking Tape

9 Analytical Balance

10. Foil Paper

11. Stir Rods

12. Disposable Latex Hand Gloves

13. Universal Bottles

14. Thermostatic Water Bath

15. Spectrometer

16. Incubator

17. Stove

3.4 Sample collection

The Moringa leaves were obtained from Ringroad market, Benin City, Nigeria. The plants were identified and authenticated by Prof. H.A. Akinnibosun in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria. The specimen was deposited at the University of Benin Herbarium with voucher UBH-M340.

3.5 Sample preparation

The collected leaves were washed with tap water, then with distilled water, to remove any form of contaminants. The leaves were then cut into small pieces and weighed to 5kg with an analytical balance. It was homogenized in 100 mL of methanol using a mortar and pestle till it was finely smooth liquid. The sample was then turned into a 100 flat flat-bottom flask and then kept at room temperature till it was required for analysis.

Figure 3.1: Weighing the Moringa leaves using a digital weighing balance.

Figure 3.2: Homogenizing the Moringa leaves

3.6 DETERMINATION OF *IN VITRO* ANTIOXIDANT ACTIVITY

3.6.1 Ferric Reducing Antioxidant Power (FRAP) Assay

The Ferric Reducing Antioxidant Power (FRAP) assay was carried out using a modified method of Benzie and Strain (1996). To 1.5ml of freshly prepared FRAP solution (25ml of 300mM), acetate buffer pH 3.6, 2.5ml of 10mM 2,4,6-tripyridyl-triazine (TPTZ) in 40mM HCl, and 2.5ml of 20mM Ferric chloride ($FeCl_3.6H_2O$) solution was added to 1ml of the extracts at concentrations of 100-600uM. The reaction mixtures were incubated at 37 °C for 30 minutes, and the increase in absorbance at 593nm was measured. $FeSO_4$ was used for the calibration curve and ascorbic acid served as the positive control. FRAP values (expressed as mM.Fe(ų/g) of the extract) for the extracts were then extrapolated from the standard curve.

3.6.2 Determination of Total Antioxidant Capacity

Total antioxidant activity was estimated by phosphomolybdenum assay (Prieto *et al*., 1999). The method is based on the reduction of molybdenum (IV) to molybdenum (V) by the extract and the subsequent formation of a green phosphate/molybdenum (V) complex at acid pH. Three millilitres (3mL) of the extracts (1mg/mL) were added to 1ml molybdate reagent solution. These tubes were incubated at 95°C for 90min. After incubation, the tubes were normalized to room temperature for 20-30minutes and the absorbance of the reaction mixture was measured at 695nm. Ascorbic acid was used as the standard.

3.6.3 Estimation of Diphenyl-2-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging capacity of the extract against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by a slightly modified method of Brand-Williams *et al.* (1995). Briefly, 0.5ml of 0.3mM DPPH solution in methanol was added to 2ml of various concentrations (0.2-1.0mg/mL) of the extracts. The reaction tubes were shaken and incubated for 15min at room temperature in the dark; absorbance was read at 517nm. All tests were performed in triplicate. Ascorbic acid was used as a standard control, with similar concentrations to the test samples prepared. A blank containing 0.5mL of 0.3mM DPPH and 2mL methanol was prepared and treated as the test samples.

The radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) = $[(A_0 - A_1) / (A_0)] \times 100$,

Where A_0 was the absorbance of DPPH radical + Methanol.

A_1 was the absorbance of DPPH radical + sample extract or standard.

The 50% inhibitory concentration value (IC_{50}) was calculated as the effective concentration of the extract that is required to scavenge 50% of the DPPH free radicals.

3.6.4 Estimation of Nitric Oxide Scavenging Ability

The method of Garret (1964) was used to determine the nitric radical scavenging ability of the extracts.

Principle

Nitric oxide is generated from sodium nitroprusside in aqueous solution at physiological pH with oxygen to produce nitrite ions, which is measured by the Griess reaction. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite.

Procedure

A volume of 2ml of 10mM sodium nitroprusside was prepared in phosphate buffer saline (pH 7.4) and was mixed with 0.5ml of the extract at various concentrations ranging from 10 to 200ug/ml and ascorbic acid at various concentrations ranging from 10 to 200ug/ml. The mixture was incubated at 25 °C. After 150 min, 0.5ml of the incubated solution was withdrawn and mixed with 0.5ml of Griess reagent[1.0ml sulfanilic acid reagent(0.33% prepared in 20% glacial acetic acid at room temperature for 5min with 1ml of napthylethylene diamine dihydrochloride (0.1%w/v)]. The mixture was incubated at room temperature for 30 minutes, followed by the measurement of absorbance read at 540nm.

CHAPTER 4

4.0 RESULTS

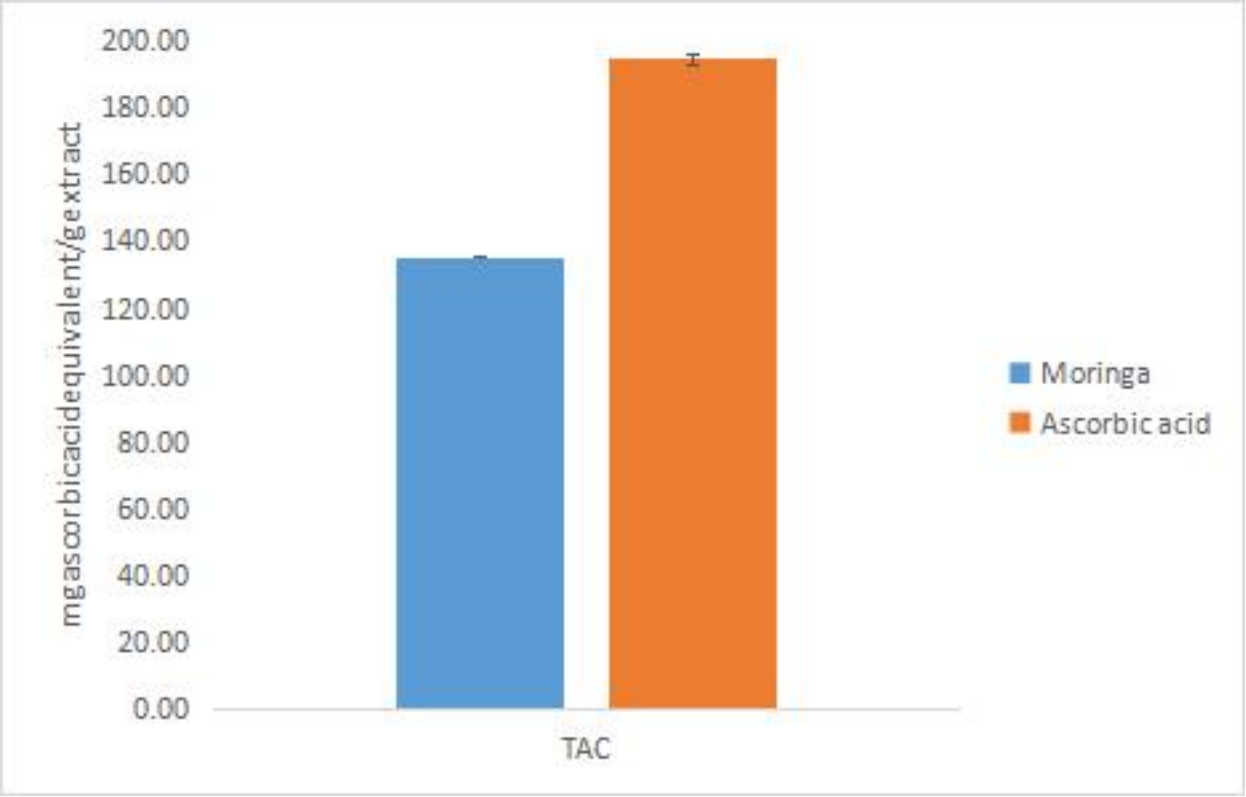

Figure 4.1: Total antioxidant capacity at different concentrations of ascorbic acid and methanol extracts of *Moringa oleifera*

Y-axis % inhibition of TAC

X-axis concentration (ug/ml)

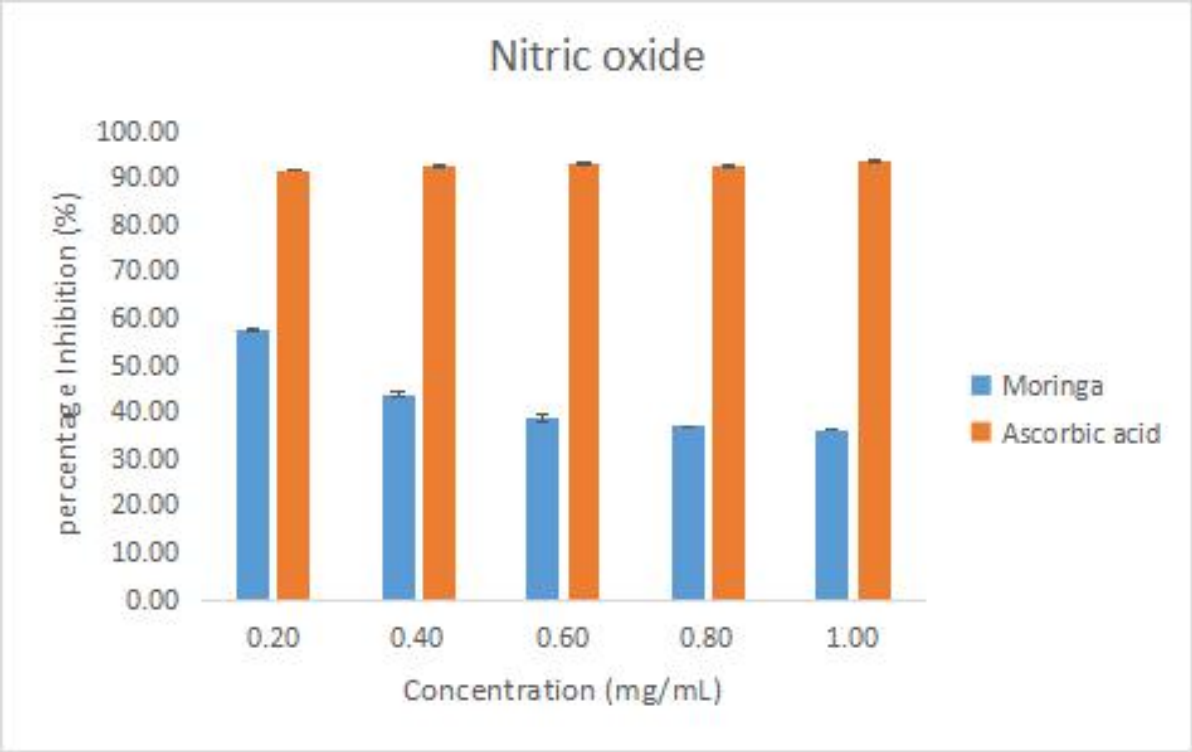

Figure 4.2: Nitric oxide at different concentrations of ascorbic acid and methanol extracts of *Moringa oleifera*

Y-axis % inhibition of Nitric oxide

X-axis concentration (ug/ml)

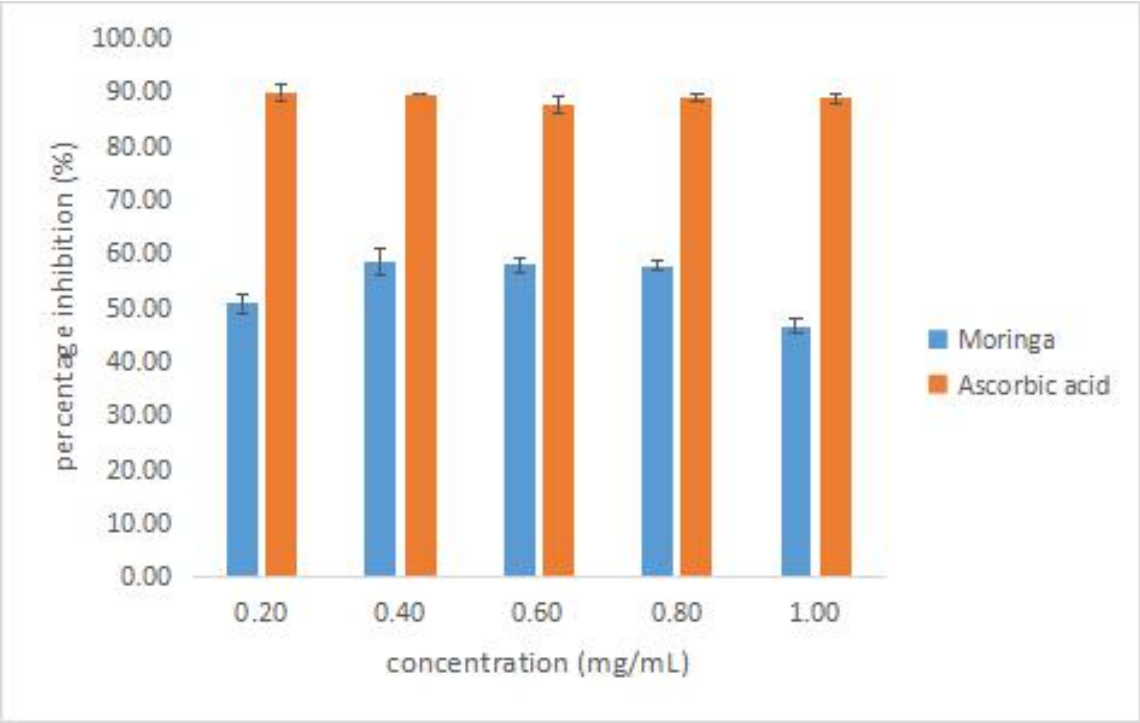

Figure 4.3: Ferric reducing antioxidant power scavenging activity at different concentrations of ascorbic acid and methanol extracts of M*oringa oleifera*

Y- axis % inhibition of FRAP

X- axis concentration (ug/ml)

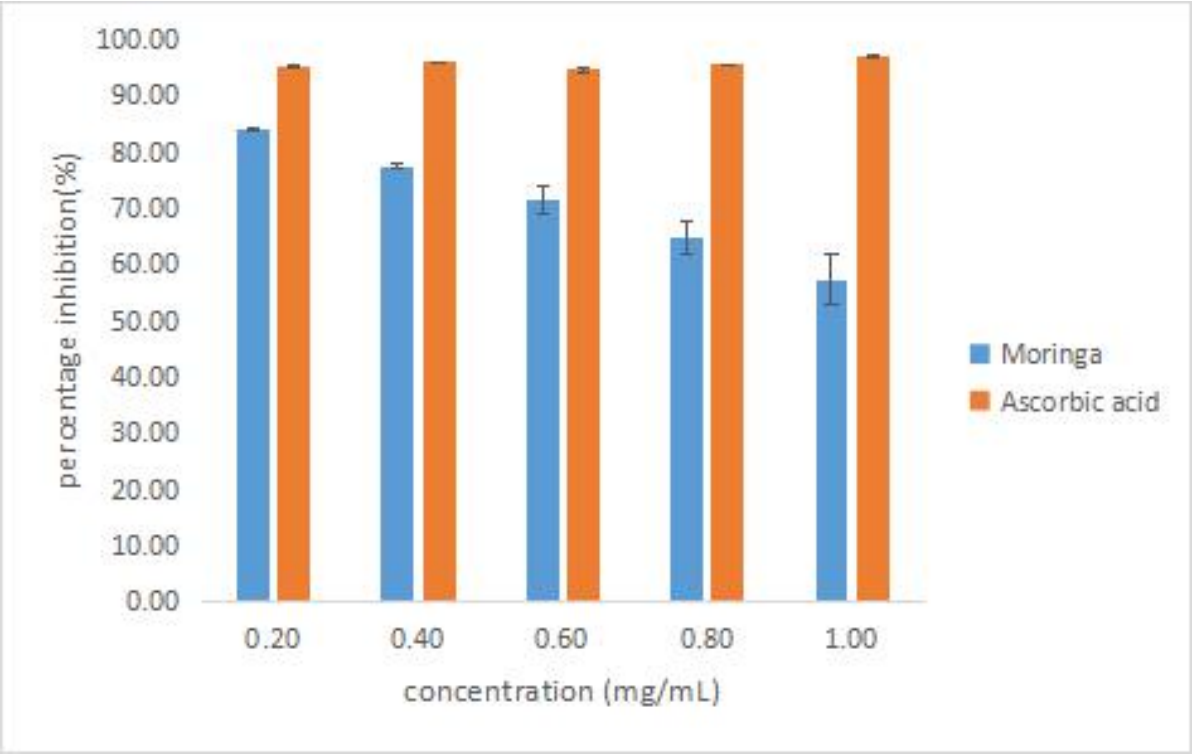

Figure 4.4: 2-Diphenyl-1-picryl-hydrazyl scavenging activity at different concentrations of ascorbic acid and methanol extracts of *Moringa oleifera*

Y-axis % inhibition of DPPH

X-axis concentration (ug/ml)

CHAPTER 5

5.0 DISCUSSION

This research investigated the in vitro antioxidant properties of M*oringa oleifera* leaf extract in relation to its potential role in the management of oxidative stress. Standard antioxidant assays were carried out, and the results were compared with ascorbic acid, which served as the reference standard. The results provide compelling evidence that M*oringa oleifera* extract possesses significant antioxidant activity that can help mitigate oxidative stress.

In Nitric Oxide, the inhibitory concentration of M*oringa oleifera* was observed to be 57.42, which is lower compared to the standard ascorbic acid of 91.62 at 0.20mg/mL concentration. At 0.40 mg/mL concentration, the inhibitory concentration of M*oringa oleifera* was 43.75, which is lower compared to the standard ascorbic acid of 92.50. The inhibitory concentration of M*oringa oleifera* was observed to be 38.79, which is lower compared to the standard ascorbic acid of 93.01 at 0.60mg/mL concentration. At 0.80mg/ml concentration, the inhibitory concentration of M*oringa oleifera* was observed to be 36.96, which is lower compared to the standard ascorbic acid of 92.48. At 1.0mg/mL, the inhibitory concentration of M*oringa oleifera* was 36.34

compared to the standard ascorbic acid of 93.53. At 0.2mg/mil the inhibitory value of nitric oxide was observed to be highest when compared to the various concentrations of 0.4,0.6,0.8, and 1.0mg/ml.

This confirms that M*oringa oleifera* contains bioactive compounds capable of reducing nitric oxide radicals, helping to suppress oxidative stress and inflammation.

The Ferric Reducing Acid Potential (FRAP) determines the ability of antioxidants to donate electrons, reducing Fe^{3+} (ferric ions) to Fe^{2+} (ferrous ions).

This assay shows that the inhibitory concentration of Moringa was observed to be 50.82, which is lower compared to the standard ascorbic acid of 90.07 at 0.20mg/ml concentration. At 0.40mg/mL concentration, the inhibitory concentration of M*oringa oleifera* was observed to be 58.58, which is lower compared to the standard ascorbic acid of 89.76. At 0.60mg/mL concentration, the inhibitory concentration of M*oringa oleifera* was observed to be 58.09, which is lower compared to the standard ascorbic acid of 87.91. At 0.80mg/mL concentration, the inhibitory concentration of M*oringa oleifera* was observed to be 57.88, which is lower when compared to the standard ascorbic acid of 89.04. At 1.0mg/mL concentration, the inhibitory

concentration of M*oringa oleifera* was observed to be 46.68 ,which is lower when compared to the standard ascorbic acid of 89.04. At 0.40mg/mL, the inhibitory value of Ferric reducing acid potential (FRAP) was observed to be highest when compared to the various concentrations of 0.20,0.60,0.80 and 1.0mg/mil. This shows M*oringa oleifera* possesses strong electron-donating antioxidants effective in neutralizing oxidizing agents and preventing cellular damage.

DPPH assay evaluates the ability of antioxidants to donate electrons or hydrogen atoms to neutralize free radicals. 2-Diphenyl-1-picryl-hydrazyl (DPPH) assay shows the inhibitory concentration of M*oringa oleifera* was observed to be 84.21, which is lower compared to the standard ascorbic acid of 95.51 at 0.20mg/mL concentration. At 0.40mg/mL concentration, the inhibitory concentration of M*oringa oleifera* was observed to be 77.63, which is lower compared to the standard ascorbic acid of 96.08. At 0.60mg/mL concentration, the inhibitory concentration of M*oringa oleifera* was observed to be 71.69, which is lower compared to the standard ascorbic acid of 94.73. At 0.80mg/mL concentration, the inhibitory concentration of M*oringa oleifera* was observed to be 64.9,7, which is lower compared to the standard ascorbic acid of 95.59. At 1.0mg/mL concentration, the inhibitory concentration of

M*oringa oleifera* is 57.44, which is lower compared to the standard ascorbic acid of 97.27. At 0.20mg/mL, the inhibitory value of 2-Diphenyl 1-picryl-hydrazyl (DPPH) was observed to be the highest when compared to the various concentrations of 0.40,0.60,0.80, and 1.0mg/mL. This confirms that Moringa contains strong hydrogen-donor antioxidants that can neutralise free radicals and prevent oxidative degradation of biomolecules.

The Total antioxidant capacity of M*oringa oleifera* was calculated as 135.51+_ 0.07 was lower compared to the standard ascorbic acid, 194.71. Although the Total antioxidant capacity (TAC) of the Moringa extract was lower than that of the standard ascorbic acid, this is expected because ascorbic acid is a pure, highly concentrated antioxidant, whereas Moringa is a crude plant extract containing mixed phytochemicals. Despite this difference, Moringa still exhibited significant antioxidant activity, confirming its potential in the management of oxidative stress.

Given its demonstrated antioxidant activity, promoting Moringa as part of the everyday diet, whether as fresh leaves, powder, tea, or incorporated into meals, can significantly improve antioxidant intake, especially in communities with limited access to expensive synthetic supplements.

The results demonstrate that M*oringa oleifera* leaf extract contains strong antioxidant constituents capable of scavenging free radicals and reducing oxidative stress. Although its activity is lower than pure ascorbic acid, the extract still exhibits robust antioxidant capacity, confirming its relevance in the management of oxidative stress. This supports the use of Moringa in traditional and modern medicine for preventing oxidative-stress-related diseases such as inflammation, cardiovascular disorders, diabetes, and degenerative conditions.

5.1 CONCLUSION

This study has demonstrated that M*oringa oleifera* leaf extract possesses significant in vitro antioxidant activity and can be used in in vivo studies to confirm its potential role in the management of oxidative stress. Using three standard antioxidant assays, Nitric Oxide scavenging, Ferric Reducing Antioxidant Power (FRAP), and DPPH free radical scavenging, the extract showed considerable ability to neutralize free radicals, donate electrons, and inhibit oxidative reactions.

Overall, the results indicate that M*oringa oleifera* is a valuable natural source of antioxidants and may serve as an affordable, accessible, and effective therapeutic supplement for reducing oxidative damage and promoting general health.

REFERENCES

Analytical Methods Used in Determining Antioxidant Activity: A Review. (2021) – PubMed. PubMed

Anwar, F., Latif, S., Ashraf, M., & Gilani, A. H. (2007). Moringa oleifera: A food plant with multiple medicinal uses. *Phytotherapy Research, 21*(1), 17–25.

Chumark, P., et al. (2008). The in vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. leaves. *Journal of Ethnopharmacology, 116*(3), 439–446.

Dhakar R., Pooniya B., Gupta M., Maurya S., Bairwa N. Sanwarmal (2011). Moringa: The herbal gold to combat malnutrition. *Chron. Young Sci., 2*, 119.

Fahey, J. W. (2005). *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *Trees for Life Journal, 1*(5), 1–15.

Fejér J., Kron I., Pellizzeri V., Pluchtová M., Eliašová A., Campone L., Gervasi T., Bartolomeo G., Cicero N., Babejová A., et al. (2019). First report on evaluation of basic nutritional and antioxidant properties of *moringa oleifera* lam. from caribbean island of saint lucia. *Plants, 8*, 537. doi: 10.3390/plants8120537.

Fokwen V.F., Tsafack H.D., Touko B.A.H., Djopnang D., Afeanyi T.A., Kong A.T., Djikeng F.T., Womeni H.M. (2019). Nutrients Composition, Phenolic Content and Antioxidant Activity of Green and Yellow *Moringa oleifera* leaves. *J. Food Stab., 1*, 46–56.

Fuglie L.J. (2001). The Miracle Tree: The Multiple Attributes of *Moringa*. Church World Service; New York, NY, USA: West Africa Regional Office; Washington, DC, USA: National Council of the Churches of Christ in the United States of America; Church World Service; Agricultural TC; Rural Cooperation.

Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness, 5*(2), 49–56.

Gulçin İ. (2020). Antioxidants and antioxidant methods: an updated overview. *Archives of Toxicology, 94*(3), 651-715.

Jongrungruangchok S., Bunrathep S., Songsak T. (2010). Nutrients and Minerals Content of Eleven Different Samples of *Moringa Oleifera* Cultivated in Thailand. *J. Health Res., 24*, 123–127.

Kasolo, J. N., Bimenya, G. S., Ojok, L., Ochieng, J., & Ogwal-Okeng, J. W. (2010). Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. *Journal of Medicinal Plants Research, 4*(9), 753–757.

Moharram H. A., Youssef M. M. (2014). Methods for Determining the Antioxidant Activity: A Review. *Alexandria Journal of Food Science & Technology, 11*(1), 31-42.

Moyo B., Masika P.J., Hugo A., Muchenjel V. (2011). Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. *Afr. J. Biotechnol., 10*, 12925–12933.

Nwachukwu I. D., Amini Sarteshnizi R., Udenigwe C. C., Aluko R. E. (2021). "A Concise Review of Current In Vitro Chemical and Cell-Based Antioxidant Assay Methods." *Molecules, 26*(16):4865.

Offor I.F., Ehiri R., Njoku C. (2014). Proximate Nutritional Analysis and Heavy Metal Composition of Dried *Moringa Oleifera* Leaves from Oshiri Onicha L.G.A, Ebonyi State, Nigeria. *IOSR J. Environ. Sci. Toxicol. Food Technol., 8*, 57–62.

Sánchez-Machado D.I., Núñez-Gastélum J.A., Reyes-Moreno C., Ramírez-Wong B., López-Cervantes J. (2009). Nutritional quality of edible parts of *Moringa oleifera*. *Food Anal. Methods, 3*, 175–180.

Siddhuraju, P., & Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of *Moringa oleifera* leaves. *Journal of Agricultural and Food Chemistry, 51*(8), 2144–2155.

Sreelatha, S., & Padma, P. R. (2009). Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant Foods for Human Nutrition, 64*(4), 303–311.

Verma, A. R., Vijayakumar, M., Mathela, C. S., & Rao, C. V. (2009). In vitro and in vivo antioxidant properties of different fractions of *Moringa oleifera* leaves. *Food and Chemical Toxicology, 47*(9), 2196–2201.