IN VITRO ANTIOXIDANT ACTIVITY OF *Azadirachta indica* (DOGOYARO) LEAF EXTRACT AND ITS POTENTIAL ROLE IN OXIDATIVE STRESS MANAGEMENT

BY

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CERTIFICATION

This is to certify that this project work was carried out by OLUMOKOR KEHINDE PETER with matriculation number BMS2209747 of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin city, in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) degree in Medical Biochemistry

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DEDICATION

I dedicate this work to God Almighty, my source of strength,inspiration, wisdom, knowledge and understanding and to my lecturers who have taught me up to this point in my academic pursuit, equipping me with knowledge for both self and societal development.

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I would like to express my sincere appreciation to everyone who contributed to the success of this project.

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ABSTRACT

The in vitro antioxidant activity of methanolic leaf extract of *Azadirachta indica* (Dogoyaro) was evaluated to determine its potential role in oxidative stress management. Oxidative stress arises from excess reactive oxygen species, contributing to various chronic diseases and increasing the need for safe, plant-derived antioxidants. The extract was analysed using four standard assays: DPPH radical scavenging, ferric reducing antioxidant power (FRAP), nitric oxide scavenging, and total antioxidant capacity (TAC). Results revealed notable antioxidant properties, with the highest DPPH activity observed at 0.20 mg/mL. Nitric oxide inhibition and reducing power also showed moderate effectiveness, particularly at lower concentrations. Although antioxidant activity remained lower than that of ascorbic acid across all assays, the extract demonstrated meaningful free-radical-neutralizing ability and electron-donating capacity. The TAC value further indicated the presence of bioactive phytochemicals contributing to overall antioxidant strength. Overall, the findings support the potential of *Azadirachta indica* leaves as a natural antioxidant source capable of contributing to the management of oxidative stress–related conditions

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The human body continuously generates reactive oxygen species (ROS) and free radicals during normal physiological activities such as cellular respiration, immune responses, and metabolic reactions (Halliwell & Gutteridge, 2015). Under normal conditions, these reactive species are neutralized by endogenous antioxidant defense systems, thereby maintaining cellular balance. However, when ROS production exceeds the body's antioxidant capacity, the resulting oxidative stress can damage lipids, proteins, and nucleic acids, leading to cellular dysfunction and contributing to the development of chronic diseases including diabetes, cardiovascular disorders, cancer, and neurodegenerative conditions (Lobo et al., 2010; Valko et al., 2007).

Oxidative stress is recognized as a key biochemical mechanism underlying various forms of tissue injury. Antioxidants play an essential protective role by scavenging free radicals or preventing their formation, thereby reducing oxidative damage and helping to maintain physiological stability (Pham-Huy et al., 2008). Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been employed in pharmaceutical and food industries to slow oxidative deterioration. However, their long-term use raises concerns due to reported safety and toxicity issues (Pokorný, 2019; Ebrahimzadeh et al., 2010).

These concerns have encouraged a shift towards antioxidant sources obtained from plants, which are generally considered safer, more accessible, and rich in naturally occurring compounds that contribute to antioxidant defenses (Tungmunnithum et al., 2018; Cadenas & Davies, 2000). Medicinal plants have therefore become increasingly important as promising alternatives for managing oxidative stress and related disorders.

Dogoyaro (Azadirachta indica), commonly known as neem, is an evergreen medicinal plant widely distributed in tropical and subtropical regions, including West Africa. It has been extensively used in traditional medicine for managing ailments such as malaria, inflammation, fever, and various infections (Subapriya & Nagini, 2005). Scientific reports show that extracts from A. indica possess biological activities including antimicrobial, anti-inflammatory, and antioxidant effects (Singh et al., 2014; Kharwar et al., 2012). These properties make the plant a promising natural candidate for combating oxidative stress and promoting health.

The antioxidant activity of A. indica leaves has attracted research attention due to their ability to neutralize free radicals and reduce oxidative damage (Chowdhury et al., 2013). Assessing the in vitro antioxidant capacity of Dogoyaro leaf extracts provides a scientific basis for verifying its traditional use and understanding its potential role in managing oxidative stress–related conditions (Lobo et al., 2010; Prieto et al., 1999).

1.2 Statement of the Problem

The rise in oxidative stress–associated diseases has become a global health concern. Increased exposure to environmental toxins, poor nutritional habits, and lifestyle factors contribute to elevated ROS levels, overwhelming the body's antioxidant defenses (Valko et al., 2007). While synthetic antioxidants are effective, their potential toxicity limits their long-term use (Pokorný, 2019).

Although Dogoyaro (Azadirachta indica) is widely used in traditional medicine, there is still limited scientific evaluation on how its antioxidant properties contribute to reducing oxidative stress under laboratory conditions (Subapriya & Nagini, 2005). Understanding its antioxidant activity through validated in vitro assays is essential for establishing its relevance in oxidative stress management.

1.3 Justification of the Study

Given the central role of oxidative stress in many chronic illnesses, identifying safe and effective natural antioxidants has become increasingly important (Lobo et al., 2010). Dogoyaro is commonly available, inexpensive, and historically valued for medicinal purposes. Despite this, scientific evidence supporting its antioxidant capacity is insufficient and inconsistent across studies (Kharwar et al., 2012).

This study is justified by the need to provide laboratory-based evidence on the antioxidant potential of Dogoyaro leaves, thereby supporting their traditional use and contributing to the development of natural antioxidant therapies (Pham-Huy et al., 2008).

1.4 Aim of the Study

To evaluate the in vitro antioxidant activity of Dogoyaro (Azadirachta indica) leaf extract and determine its potential role in oxidative stress management.

1.5 Objectives of the Study

1. To determine the DPPH radical scavenging activity of Dogoyaro leaf extract.
2. To evaluate the ferric reducing antioxidant power (FRAP) of the extract.
3. To assess the total antioxidant capacity (TAC) using the phosphomolybdenum assay.
4. To determine the nitric oxide scavenging ability of the extract.
5. To relate the antioxidant activity of the extract to its potential usefulness in oxidative stress management.

1.6 Significance of the Study

This study provides scientific insight into the antioxidant potential of Azadirachta indica leaves, supporting their traditional use as a natural remedy for conditions associated with oxidative damage. Establishing its antioxidant capacity contributes to current knowledge in phytomedicine and may promote the use of indigenous plant resources in managing oxidative stress–related diseases (Tungmunnithum et al., 2018; Pham-Huy et al., 2008).

CHAPTER 2

LITERATURE REVIEW

2.1 Concept of Oxidative Stress

Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and the capacity of the body's antioxidant defense system to neutralize them (Halliwell & Gutteridge, 2015). Under normal physiological conditions, ROS such as hydrogen peroxide, hydroxyl radical, and superoxide are generated as natural by-products of respiration, metabolism, and immune response. Moderate levels of ROS are essential in cellular signaling and host defense mechanisms (Valko et al., 2007).
However, when ROS production exceeds antioxidant capacity, oxidative stress occurs, leading to damage of lipids, proteins, and DNA. This process contributes significantly to chronic illnesses including diabetes mellitus, atherosclerosis, neurodegenerative diseases, and certain cancers (Lobo et al., 2010; Pham-Huy et al., 2008).

2.2 Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)

ROS and RNS are highly reactive derivatives of oxygen and nitrogen metabolism. Key ROS include superoxide anions, hydroxyl radicals, and hydrogen peroxide, while major RNS include nitric oxide (NO) and peroxynitrite (Iqbal et al., 2006).
These species can alter cell membranes, initiate lipid peroxidation, and disrupt cellular integrity (Aruoma, 1998). Sources include mitochondrial electron leakage, environmental pollutants, inflammatory responses, and xenobiotic metabolism (Valko et al., 2007). Persistent accumulation of ROS/RNS is a major driving force in oxidative and nitrosative damage.

2.3 Antioxidants and Their Biological Importance

Antioxidants are molecules that prevent oxidative deterioration by scavenging free radicals, donating electrons, inhibiting lipid peroxidation, or enhancing endogenous defense enzymes (Cadenas & Davies, 2000). They exist as:

Endogenous antioxidants: catalase, glutathione, superoxide dismutase

Exogenous antioxidants: obtained from food, medicinal plants, and supplements (Lobo et al., 2010)

Antioxidants are crucial in maintaining cellular stability and preventing oxidative stress-related pathologies. Plant-derived antioxidants are especially important due to their accessibility, low toxicity, and historical use in traditional medicine (Tungmunnithum et al., 2018).

2.4 Medicinal Plants as Natural Sources of Antioxidants

Medicinal plants remain crucial sources of natural antioxidant agents. These plants contain compounds capable of neutralizing ROS, improving cellular defense, and reducing oxidative injury (Pokorný, 2019).
Research interest continues to grow due to concerns about synthetic antioxidants like BHA and BHT and their potential long-term toxicity (Pham-Huy et al., 2008). Plants used in African and Asian traditional medicine have been studied for their role in oxidative stress mitigation, including *Azadirachta indica*.

2.5 The Plant: *Azadirachta indica* (Dogoyaro/Neem)

2.5.1 Taxonomy of *Azadirachta indica*

Rank	Classification
Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Sapindales
Family	Meliaceae
Genus	*Azadirachta*
Species	*Azadirachta indica*

Fig. 2.5 The image shows the plant Aloe vera
Source: Healthline articles

2.5.2: Botanical Description

Azadirachta indica is a large evergreen tree that grows up to 15–20 meters tall. It has compound pinnate leaves, serrated leaflets, and a straight trunk with a wide crown. Flowers are small, white, and fragrant, occurring in clusters, while fruits are smooth olive-like drupes containing a single seed (Subapriya & Nagini, 2005). The tree thrives in tropical climates and is widely distributed in West Africa, including Nigeria, where it is commonly called *Dogoyaro*.

2.5.3: Ethnomedicinal Uses

Neem leaves, bark, and seeds are widely used for treating malaria, fever, skin infections, inflammation, gastrointestinal disorders, and diabetes (Kharwar et al., 2012). Its popularity in traditional medicine is due to its broad therapeutic potential and minimal toxicity.

2.6 Oxidative Stress and Disease Development

Oxidative stress plays a foundational role in multiple disease pathways. Excess ROS contributes to:

Diabetes mellitus: oxidative damage to pancreatic β-cells (Valko et al., 2007)

Cardiovascular disease**:** endothelial dysfunction and LDL oxidation

Cancer**:** DNA mutation and genomic instability

Neurodegenerative disorders**:** neuronal cell damage and mitochondrial impairment (Lobo et al., 2010)

Understanding plant-based antioxidants is therefore vital for mitigating oxidative stress in such conditions.

2.7: Mechanisms of Antioxidant Action

Antioxidants act through several biological mechanisms:

Free radical scavenging – donating electrons/hydrogen atoms (Brand-Williams et al., 1995)

Reducing power – converting $Fe^{3+} \rightarrow Fe^{2+}$ (Benzie & Strain, 1996)

Metal ion chelation – binding pro-oxidant metal ions

Nitric oxide inhibition – reducing nitrite formation via Griess reaction (Iqbal et al., 2006)

Enhancing endogenous enzymes – catalase, SOD, glutathione reductase (Aruoma, 1998)

These mechanisms justify the use of multiple antioxidant assays in evaluating plant extracts.

2.8: In-Vitro Antioxidant Assays Used in Evaluation

2.8.1 DPPH Radical Scavenging Assay

Assesses ability to neutralize the stable purple DPPH radical by electron or hydrogen donation (Brand-Williams et al., 1995).

2.8.2 FRAP (Ferric Reducing Antioxidant Power)

Evaluates reducing capacity by measuring the $Fe^{3+} \rightarrow Fe^{2+}$ conversion (Benzie & Strain, 1996).

2.8.3 Nitric Oxide Scavenging Assay

Measures inhibition of nitrite formation from s odium nitroprusside using Griess reagent (Iqbal et al., 2006).

2.8.4 Phosphomolybdenum Total Antioxidant Capacity

Assesses overall antioxidant potency via reduction of Mo(VI) → Mo(V) (Prieto et al., 1999).

2.9 Summary of Literature Review

Although neem (*Azadirachta indica*) has been widely researched, limited studies evaluate multiple antioxidant mechanisms in a standardized experimental framework. Few studies also link these antioxidant properties specifically to oxidative stress mitigation.
This research fills these gaps by assessing **four complementary assays**—DPPH, FRAP, nitric oxide scavenging, and total antioxidant capacity—to provide a clearer scientific basis for the antioxidant potential of Dogoyaro leaves.

CHAPTER THREE

MATERIALS AND METHODS

3.1: MATERIALS

3.1.1 : REAGENTS

- 300 mM acetate buffer (pH 3.6)
- 10 mM TPTZ in 40 mM HC
- 20 mM $FeCl_3 \cdot 6H_2O$
- $FeSO_4$ (standard)
- Ascorbic acid (control/standard)
- Molybdate reagent
- 0.3 mM DPPH in methanol
- Methanol
- 10 mM sodium nitroprusside
- Phosphate buffer saline (PBS), pH 7.4
- Sulfanilic acid reagent (0.33% in 20% acetic acid)
- Naphthylethylenediamine dihydrochloride (0.1%)
- Dogoyaro (Neem) leaf extract

3.1.2 Apparatus / Equipment

- Test tubes
- Pipettes / micropipettes
- Beakers
- Volumetric flasks
- Measuring cylinders
- Analytical balance
- Water bath
- Ice bath
- Incubator
- Spectrophotometer (517 nm, 540 nm, 593 nm, 695 nm)
- Stopwatch

3.2: METHODS

3.21: EXTRACTION PROCEDURE

The extraction of the bioactive constituents from the Dogoyaro (Azadirachta indica) leaves was carried out using methanol as the solvent, following a modified cold maceration procedure similar to the extraction method applied for Moringa leaves in related studies. Freshly collected Dogoyaro leaves were first washed thoroughly under running tap water and rinsed with distilled water to remove dust and contaminants. The leaves were air-dried at room temperature for 7–10 days to prevent degradation of thermolabile phytochemicals. The dried leaves were then pulverized into a fine powder using a clean electric grinder (Binatone Model BLG-450, China).

A measured quantity of the powdered sample (150 g) was placed into an amber extraction bottle, and 500 mL of analytical-grade methanol (≥99.8%, Sigma-Aldrich, Germany; Batch No. MA-2024-17) was added. The mixture was kept in a tightly sealed container and macerated for 72 hours with intermittent shaking three times daily to enhance solvent penetration and solute diffusion. After the maceration period, the mixture was filtered first through muslin cloth and then through Whatman No. 1 filter paper to obtain a clear methanolic filtrate.

The methanol filtrate was concentrated using a rotary evaporator (Büchi Rotavapor R-300, Switzerland) under reduced pressure at 40°C to completely remove the solvent without destroying heat-sensitive compounds. The resulting crude methanol extract was transferred into sterile, airtight universal bottles and stored in a refrigerator at –4°C until required for antioxidant assays.

3.3: IN-VITRO ANTIOXIDANT ASSAYS

3.3.1: Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power (FRAP) of the methanolic extract of *Azadirachta indica* leaves was determined using the method of Benzie and Strain (1996), with slight modification. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ prepared in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in the ratio 10:1:1.

A volume of 1 mL of the extract at concentrations ranging from 100–600 µg/mL was added to 1.5 mL of the FRAP reagent, mixed thoroughly, and incubated at 37 °C for 30 minutes. The increase in absorbance was measured at 593 nm using a UV-visible spectrophotometer.

Ferric sulphate ($FeSO_4$) was used to generate the calibration curve, while ascorbic acid served as the positive control. The FRAP values were calculated from the standard curve and expressed as mM Fe^{2+} equivalents per gram of extract.

3.3.2: Total Antioxidant Capacity (Phosphomolybdenum Assay)

Total antioxidant activity was assessed using the phosphomolybdenum method described by Prieto et al. (1999). One milligram per milliliter (1 mg/mL) of the extract was mixed with 1 mL of molybdate reagent solution (containing sulphuric acid, sodium phosphate, and ammonium molybdate).

The reaction mixture was incubated at 95 °C for 90 minutes in a water bath. After cooling to room temperature, the absorbance of the resulting green complex was recorded at 695 nm.

Ascorbic acid was used as the standard antioxidant, and results were expressed as ascorbic acid equivalents (AAE).

3.3.3: DPPH Radical Scavenging Assay

The free radical scavenging ability of the extract was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. A 0.3 mM solution of DPPH in methanol was prepared and protected from light.

A volume of 0.5 mL of the DPPH solution was added to 2 mL of the extract at varying concentrations (0.2–1.0 mg/mL). The reaction mixture was vortexed gently and incubated for 15 minutes at room temperature in the dark. Absorbance was measured at 517 nm against methanol as blank.

Ascorbic acid served as the standard antioxidant. The percentage radical scavenging activity was calculated using:

DPPH Scavenging (%)=A0−A1A0×100\text{DPPH Scavenging (\%)} = \frac{A_0 - A_1}{A_0} \times 100DPPH Scavenging (%)=A0 A0 −A1 ×100

where A0A_0A0 is the absorbance of DPPH + methanol and A1A_1A1 is the absorbance of DPPH + extract.
The IC_{50} value (concentration required to scavenge 50% of DPPH radicals) was extrapolated from the inhibition curve.

3.3.4: Nitric Oxide (NO) Scavenging Assay

Nitric oxide scavenging activity of the extract was evaluated based on the Griess reaction. Sodium nitroprusside (10 mM) in phosphate buffer (pH 7.4) was incubated with different concentrations of the extract (10–200 µg/mL) at 25 °C for 150 minutes.

A 0.5 mL portion of the incubated mixture was treated with an equal volume of Griess reagent, prepared by combining sulfanilic acid (0.33% in 20% acetic acid) with naphthylethylenediamine dihydrochloride (0.1% w/v).

The mixture was allowed to stand for 30 minutes at room temperature, after which absorbance was measured at 540 nm. Ascorbic acid was used as the reference standard.

Nitric oxide scavenging activity was expressed as percentage inhibition of nitrite formation.

CHAPTER FOUR

RESULTS

These are the results of the antioxidant assays carried out on the methanolic extract of Azadirachta indica and compares its activity with that of ascorbic acid across all parameters evaluated.

4.1: Nitric oxide scavenging activity of Dogoyaro extract and ascorbic acid across concentrations.

Table 4.1: The nitric oxide scavenging activity of the extract was assessed across different concentrations and compared with the standard antioxidant.

Conc(mg/mL)	0.20	0.40	0.60	0.80	1.00
Dogoyaro	71.28	58.14	52.60	44.18	42.34
Ascorbic acid	91.62	92.50	93.01	92.48	93.53

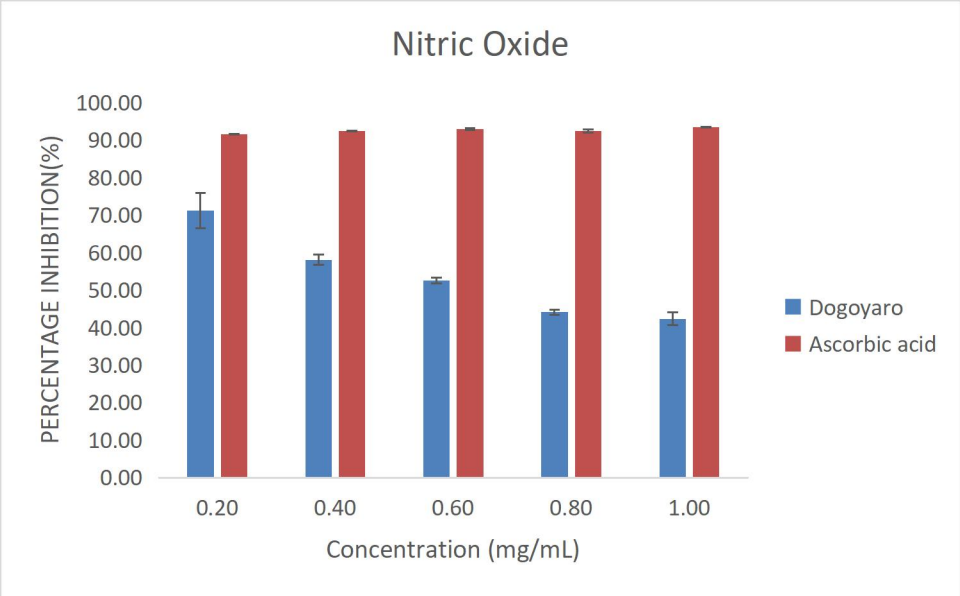

Fig 4.1 Nitric oxide scavenging activity of Dogoyaro extract and ascorbic acid across concentrations.

4.2: Ferric reducing antioxidant power (FRAP) of Dogoyaro extract compared with the standard antioxidant

Table 4.2 The ferric reducing antioxidant power (FRAP) of the extract was measured to determine its electron-donating ability relative to ascorbic acid.

Conc(mg/Ml)	0.20	0.40	0.60	0.80	1.00
Dogoyaro	52.45	48.06	46.96	45.72	43.50
Ascorbic acid	90.07	89.76	87.91	89.04	89.04

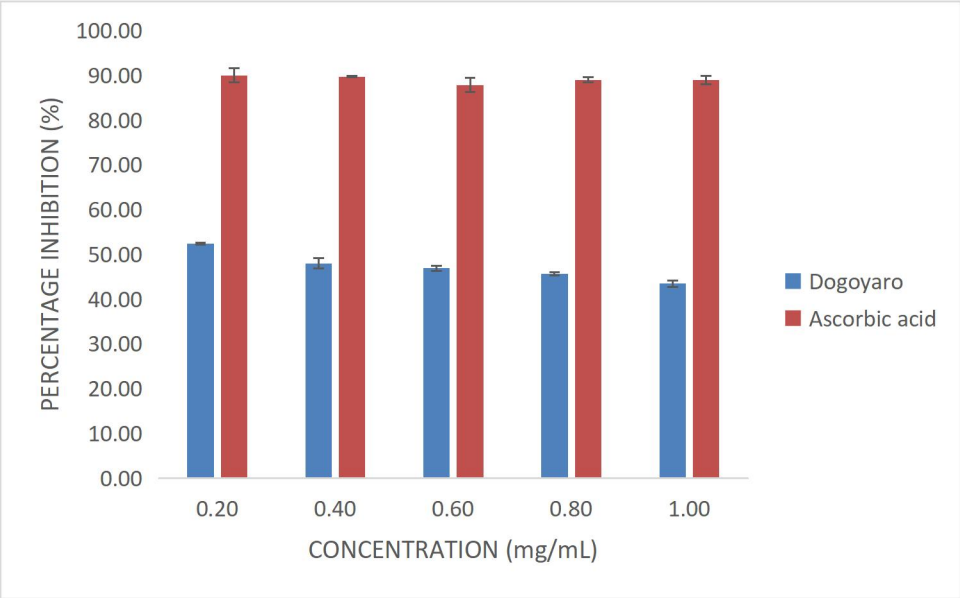

Fig 4.2 Ferric reducing antioxidant power (FRAP) of Dogoyaro extract compared with the standard antioxidant.

4.3: DPPH radical scavenging activity of Dogoyaro extract versus ascorbic acid

Table 4.3: The DPPH radical scavenging activity of the Dogoyaro extract was evaluated to determine its free-radical quenching ability in comparison with ascorbic acid.

Conc(mg/Ml)	0.20	0.40	0.60	0.80	1.00
Dogoyaro	89.69	91.08	89.20	82.98	75.24
Ascorbic acid	95.51	96.08	94.73	95.59	97.27

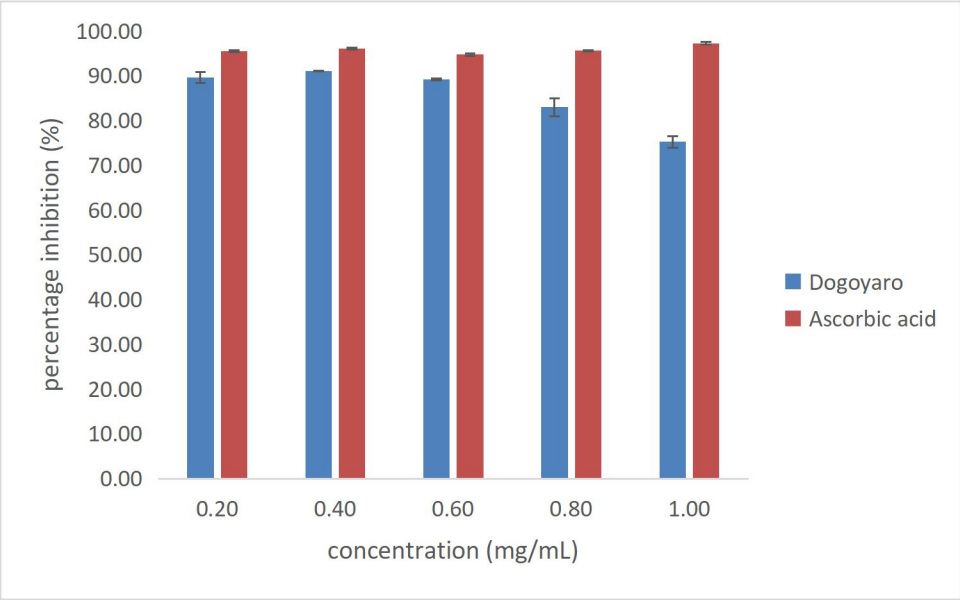

Fig 4.3 DPPH radical scavenging activity of Dogoyaro extract versus ascorbic acid.

4.4 Total antioxidant capacity (TAC) of Dogoyaro extract compared with ascorbic acid

Table 4.4: The total antioxidant capacity (TAC) of the extract was evaluated using the phosphomolybdenum assay and compared with ascorbic acid.

Dogoyaro	Ascorbic acid
137.93	194.71

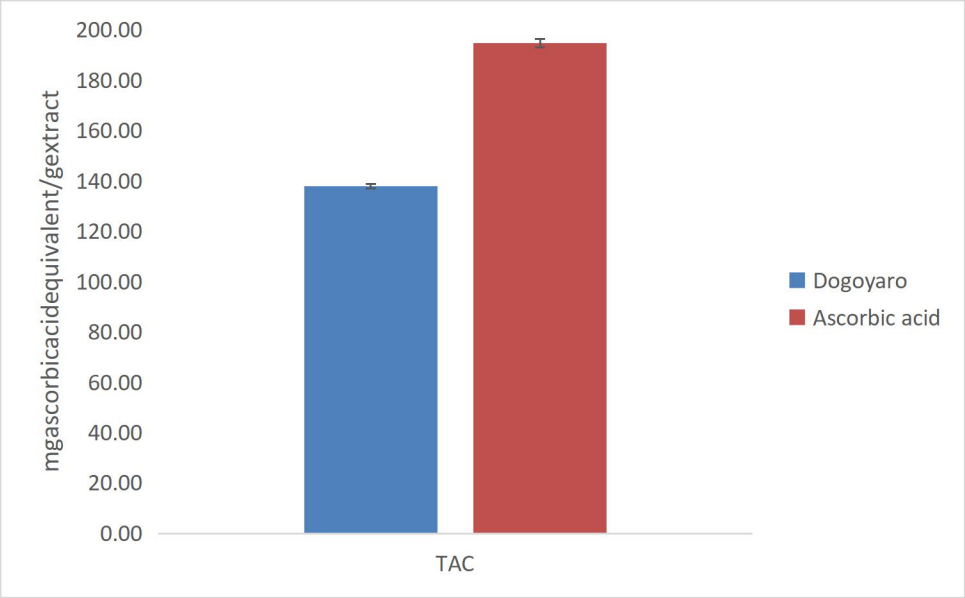

Fig 4.4 Total antioxidant capacity (TAC) of Dogoyaro extract compared with ascorbic acid.

CHAPTER FIVE

5.1 DISCUSSION

The nitric oxide scavenging activity of the methanolic extract of *Azadirachta indica* (Dogoyaro) was evaluated across concentrations of 0.20, 0.40, 0.60, 0.80, and 1.00 mg/mL, and the results were compared with ascorbic acid, which served as the standard antioxidant.

At 0.20 mg/mL, the extract exhibited the highest nitric oxide inhibitory activity **(**71.28%**)** compared to its activity at higher concentrations. However, this value was lower than the inhibition produced by ascorbic acid **(**91.62%**)** at the same concentration.

As the concentration increased to 0.40 mg/mL and 0.60 mg/mL, the percentage inhibition of Dogoyaro extract decreased to 58.14% and 52.60%**,** respectively, while ascorbic acid maintained consistently higher activity (92.50% and 93.01%).

Further increase in concentration to 0.80 mg/mL and 1.00 mg/mL resulted in even lower inhibitory values for Dogoyaro **(**44.18% and 42.34%**),** whereas the standard antioxidant remained strongly active (92.48% and 93.53%**,** respectively).

Overall, the dogoyaro extract showed moderate nitric oxide scavenging ability, with the highest activity recorded at the lowest concentration (0.20 mg/ml). however, at all concentrations tested, its inhibitory effect was significantly lower than that of ascorbic acid, indicating that while dogoyaro possesses nitric oxide scavenging properties, it is less potent than the standard antioxidant

The Ferric Reducing Antioxidant Power (FRAP) assay evaluates the ability of antioxidants to donate electrons and reduce Fe^{3+} (ferric ions) to Fe^{2+} (ferrous ions). Higher FRAP values indicate stronger reducing power.

At 0.20 mg/mL**,** the Dogoyaro extract recorded a reducing power of 52**.**45**%**, which is lower than the standard ascorbic acid value of 90.07%**.**

At **0.**40 mg/mL, Dogoyaro showed a FRAP value of 48.06%**,** still lower compared to 89.76% for ascorbic acid.

At 0.60 mg/mL, the extract produced 46.96%**,** which is lower compared to 87.91% obtained for the standard.

At 0.80 mg/mL, the Dogoyaro extract recorded 45.72%**,** also lower than the standard value of 89.04%**.**

At 1.00 mg/mL, Dogoyaro showed 43.50%**,** the lowest value among all concentrations, compared to 89.04% for the standard.

Overall, Dogoyaro exhibited its highest ferric reducing antioxidant power at 0.20 mg/mL, with a gradual decrease as the concentration increased.
Across all concentrations, ascorbic acid demonstrated significantly higher reducing power, confirming its potency as a strong reference antioxidant.

The DPPH radical scavenging assay measures the ability of antioxidants to donate hydrogen atoms or electrons to neutralize the stable DPPH radical. The methanolic extract of Dogoyaro (Azadirachta indica) showed a concentration-dependent decrease in percentage inhibition across the tested range (0.20–1.00 mg/mL).

At 0.20 mg/mL, Dogoyaro showed the highest scavenging activity (89.69%), although this value was still lower than that of the reference antioxidant, ascorbic acid (95.51%). As the concentration increased to 0.40 mg/mL and 0.60 mg/mL, the percentage inhibition slightly reduced to 91.08% and 89.20%, respectively, compared to ascorbic acid values of 96.08% and 94.73%.

Further decrease was observed at 0.80 mg/mL (82.98%) and 1.00 mg/mL (75.24%), which also remained lower than ascorbic acid (95.59% and 97.27%, respectively). This trend indicates that although Dogoyaro possesses strong DPPH scavenging ability, its activity is consistently lower than the standard across all concentrations.

The highest DPPH inhibition for Dogoyaro was observed at 0.20 mg/mL, after which the values gradually declined. This suggests that the extract contains active phytochemicals capable of

donating electrons to DPPH radicals, but their effectiveness decreases slightly at higher concentrations. Overall, the results confirm the presence of considerable antioxidant constituents in Dogoyaro, although its efficiency is lower than the pure standard antioxidant.

The Total Antioxidant Capacity assay evaluates the overall ability of a plant extract to reduce molybdenum (VI) to molybdenum (V), forming a green complex that is measured at 695 nm. Higher absorbance corresponds to stronger total antioxidant power.

From the graph, the TAC value of Dogoyaro leaf extract was approximately 138.0 mg AAE/g extract, which is lower than the TAC of the standard antioxidant, ascorbic acid **(**approximately 195.0 mg AAE/g).

This indicates that:

Dogoyaro possesses substantial antioxidant capacity, reflecting the presence of phenolics, flavonoids, and other reducing phytochemicals.

However, its total antioxidant strength is lower than that of ascorbic acid, a pure and highly potent antioxidant compound.

The difference is expected, as crude plant extracts contain mixed compounds, not all of which contribute directly to antioxidant activity.

5.2 CONCLUSION

This study provides evidence that Azadirachta indica leaves possess meaningful antioxidant activity that can contribute to reducing oxidative stress. Although its activity was lower than that of ascorbic acid, the extract showed consistent antioxidant effects across all assays, supporting its potential as a natural source of antioxidants and reinforcing its traditional use in managing conditions linked to oxidative stress.”

REFERENCES