PHYTOCHEMICAL SCREENING AND ANTIOXIDANT BIOACTIVITY CORRELATION OF *Azadirachta indica* (DONGOYARO) LEAVES: IMPLICATIONS FOR ANTIMICROBIAL AND ANTI-INFLAMMATORY MECHANISMS

BY

FAVOUR ADEOLA OLADEJO
(BMS2101439)

DEPARTMENT OF MEDICAL BIOCHEMISTRY
SCHOOL OF BASIC MEDICAL SCIENCES
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCES, B.Sc. (HONS) MEDICAL BIOCHEMISTRY, OF THE UNIVERSITY OF BENIN, BENIN CITY.

NOVEMBER, 2025

CERTIFICATION

We the undersigned hereby certify that Miss FAVOUR ADEOLA OLADEJO (BMS2101439) carried out this research in the Department of Medical Biochemistry, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry.

Signed:

……. ……

DR. L.O. AGBONTAEN. **(Date)**

(Project Supervisor)

…….. …….

DR. N. BOBBY AGUEBOR-OGIE. **(Date)**

(Head of Department)

…….. ……..

EXTERNAL EXAMINER **(Date)**

DEDICATION

This work is dedicated to the Lord almighty for his grace, insights and strength; and also to my parents and siblings for their prayers and support.

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ABSTRACT

Azadirachta indica (Dongoyaro) leaves are widely recognized in traditional medicine for its antimicrobial and anti-inflammatory properties, which are largely attributed to its diverse phytochemical composition and antioxidant capacity. However, limited studies have systematically correlated its phytochemical abundance with its antioxidant bioactivity to better explain its therapeutic mechanisms. This research aimed to profile the phytochemical constituents of *Azadirachta indica* leaves, evaluate their antioxidant properties, and determine how specific phytochemicals contribute to observed antioxidant activity.

Fresh *Azadirachta indica* leaves were subjected to qualitative and quantitative phytochemical screening to determine the presence and concentrations of major secondary metabolites. Methanolic extracts were analyzed for steroids, flavonoids, tannins, cardiac glycosides (Keller–Killiani test), saponins, and terpenoids. Antioxidant activity was assessed using standard in-vitro assays, and statistical correlation was carried out to evaluate the relationship between phytochemical concentrations and antioxidant performance.

Quantitative results showed high concentrations of flavonoids (862.21 µg/mL) and cardiac glycosides (527.75 µg/mL), while steroids (282.50 µg/mL) and terpenoids (305.48 µg/mL) were present in moderate amounts. Tannins (77.13 µg/mL) occurred in lower quantities. Antioxidant evaluation revealed strong free-radical scavenging activity, with inhibition values increasing proportionally with the concentration of flavonoids. Correlation analysis identified flavonoids as the major contributors to antioxidant capacity. These findings support the documented antimicrobial and anti-inflammatory actions of neem (Dongoyaro) leaves, as flavonoids, steroids, terpenoids, and glycosides are known to disrupt microbial membranes, modulate inflammatory pathways, and reduce oxidative stress.

Overall, this study provides biochemical justification for the therapeutic relevance of *Azadirachta indica* leaves by establishing a clear relationship between its quantified phytochemicals and antioxidant activity.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Medicinal plants have played a central role in traditional health systems for centuries, offering therapeutic benefits derived largely from their bioactive secondary metabolites. These phytochemicals including flavonoids, tannins, saponins, terpenoids, and glycosides, provide essential biological activities such as antimicrobial, antioxidant, anti-inflammatory, and anticancer effects (Burkil, 2004; Cowan, 1999). Globally, the World Health Organisation (WHO) estimates that more than 80% of the population relies on herbal medicines for primary healthcare (WHO, 2019), highlighting the continuous relevance of plant-based therapeutics.

Among these medicinal plants, *Azadirachta indica,* commonly known as neem or Dongoyaro, is one of the most extensively utilized species in traditional African and Asian medicine. Its leaves, bark, seeds, and oil are used to treat various ailments including fever, malaria, microbial infections, inflammation, and skin disorders (Kumar and Navaratnam, 2013; Subapriya and Nagini, 2005). The pharmacological importance of neem has been linked to its high content of biologically active secondary metabolites such as flavonoids, terpenoids, limonoids, and glycosides (Biswas et al., 2002).

Antioxidants play an essential role in neutralizing free radicals and preventing oxidative stress, a pathological condition associated with chronic inflammation, cellular damage, and microbial vulnerability (Halliwell and Gutteridge, 2015). Previous studies have reported that neem leaves exhibit strong antioxidant properties attributed to their rich phytochemical constituents (Nwankwo et al., 2020). However, despite the recognized therapeutic potential of neem, there is limited correlation-based research linking quantitative phytochemical abundance with antioxidant activity in order to explain how these bioactive compounds contribute to the plant's antimicrobial and anti-inflammatory mechanisms.

Establishing such correlations is critical because antioxidant activity often underlies antimicrobial and anti-inflammatory responses. For example, flavonoids can scavenge reactive oxygen species (ROS), inhibit microbial growth, and suppress the release of inflammatory mediators (Panche et al., 2016), while terpenoids and cardiac glycosides have been reported to enhance membrane disruption in pathogens and modulate cytokine signaling (Rauf et al., 2018).

Therefore, a detailed exploration of the phytochemical composition of *Azadirachta indica* leaves, alongside antioxidant assays and correlation analyses, provides a strong biochemical basis for understanding its traditional medicinal uses while contributing to scientific knowledge.

1.2 Aim of the Study

The study aims to evaluate the phytochemical composition of Azadirachta indica leaves, assess their antioxidant activity, and establish correlations between phytochemical levels and antioxidant bioactivity to explain their therapeutic relevance.

1.3 Objectives of the Study

The specific objectives are to:

- Conduct qualitative phytochemical screening of *Azadirachta indica* leaf extracts.
- Quantify major phytochemicals present in the leaves, including flavonoids, tannins, terpenoids, steroids, saponins, and cardiac glycosides.
- Determine the antioxidant activity of A. indica methanolic leaf extracts using standard in-vitro assays.
- Perform statistical correlation analysis to establish relationships between phytochemical concentrations and antioxidant activity.
- Interpret the findings in relation to the antimicrobial and anti-inflammatory mechanisms reported in literature.

1.4 Scope of the Study

The study focuses on:

- Collection and preparation of fresh Azadirachta indica leaves.
- Qualitative and quantitative screening for selected secondary metabolites.
- Antioxidant evaluation using in-vitro free radical scavenging assays.
- Correlation analysis between the quantitative phytochemicals and antioxidant activity.
- Interpretation of how these findings relate to antimicrobial and anti-inflammatory mechanisms based on existing literature.

The study is limited to leaf extracts and does not include bark, seed, or oil extracts.

1.5 Justification of the Study

Neem is a therapeutically important medicinal plant in Africa, yet there is limited biochemical evidence connecting its phytochemical richness with its antioxidant properties. Understanding these relationships is important because:

- Antioxidants contribute to the suppression of oxidative stress, a key driver of inflammation (Halliwell and Gutteridge, 2015).
- Phytochemicals such as flavonoids and terpenoids exhibit dual antioxidant and antimicrobial activity, making them significant in infectious disease control (Panche et al., 2016).
- Establishing correlation provides stronger scientific validation for traditional uses of neem.
- The findings may encourage the development of plant-based antioxidant, antimicrobial, and anti-inflammatory formulations.
- This study provides a scientific foundation that strengthens the credibility of neem as an affordable therapeutic resource

1.6 Significance of the Study

This study is significant for several scientific and practical reasons:

- Bridging a knowledge gap:

It provides empirical correlation between phytochemical abundance and antioxidant activity in *Azadirachta indica* leaves, an area where existing literature is limited, especially in Nigerian samples.

- Strengthening scientific validation of traditional medicine:

By establishing biochemical explanations for the antimicrobial and anti-inflammatory uses of neem, the study supports evidence-based integration of herbal therapies into modern healthcare (Biswas et al., 2002; Kumar and Navaratnam, 2013).

- Contribution to phytomedicine and natural product research:

The results offer valuable data for researchers interested in plant-derived antioxidants, which are increasingly studied as alternatives to synthetic compounds.

- Foundation for drug development:

Understanding which phytochemicals strongly influence antioxidant activity can guide the development of plant-based antimicrobial and anti-inflammatory agents.

- Enhanced public health relevance:

Since medicinal plants remain accessible and affordable in low-income regions, scientific validation of neem's therapeutic properties promotes safer and more informed traditional health practices.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical description of *Azadirachta indica*.

Azadirachta indica leaves, commonly called neem or Dongoyaro, is a tropical evergreen tree belonging to the family Meliaceae. It is native to the Indian subcontinent but widely distributed across Africa, including Nigeria, where it is commonly cultivated for medicinal and agricultural purposes (Biswas et al., 2002). The tree grows up to 15–20 meters in height, with pinnate leaves, white fragrant flowers, and a bitter-tasting seed kernel (Kumar & Navaratnam, 2013). Neem leaves are particularly rich in biologically active secondary metabolites such as flavonoids, terpenoids, steroids, tannins and glycosides, which account for its broad therapeutic properties (Subapriya and Nagini, 2005). These compounds are synthesized through the plant's secondary metabolism and are responsible for its diverse biological activities (Biswas et al., 2002).

2.2 Traditional Uses of Azadirachta indica.

Neem has a long history of use in traditional medicine systems across Africa, India, and Southeast Asia. In Nigerian traditional medicine, neem leaves are applied for:

- Treatment of fever and malaria by boiling leaves to make decoctions (Nwankwo et al., 2020)
- Management of skin infections and wounds using leaf pastes or extracts
- Relief of inflammatory conditions such as arthritis or swollen joints
- Dental care through chewing neem sticks or using extracts as mouth rinses to prevent microbial infection
- Digestive ailments including constipation, diarrhea, and stomach infections
- Globally, neem has also been used for antimicrobial, anti-inflammatory, antifungal, antiviral, antimalarial, and antipyretic purposes (Subapriya and Nagini, 2005; Kumar

and Navaratnam, 2013). These traditional applications are largely supported by its bioactive phytochemicals, linking ethnomedicinal knowledge to modern pharmacology.

Figure 2.1: Image of *Azadirachta indica* tree

Source: Biswas et.al.(2002)

2.3. Medicinal Importance of *Azadirachta indica*

Azadirachta indica (neem) is widely recognized for its broad spectrum of medicinal properties, which are primarily attributed to its rich phytochemical composition. The leaves, bark, seeds, flowers, and oil contain flavonoids, terpenoids, tannins, steroids, and glycosides that work through multiple biochemical pathways to exert antimicrobial, anti-inflammatory, antidiabetic, antimalarial, antioxidant, and immunomodulatory effects (Biswas et al., 2002; Subapriya & Nagini, 2005).

- Antioxidant Effects

Neem leaves contain potent antioxidant compounds such as tannins, and terpenoids. These phytochemicals neutralize reactive oxygen species (ROS), reduce lipid peroxidation, and enhance cellular defense systems (Panche et al., 2016).

The antioxidant activity of neem contributes to:

- Protection against oxidative stress
- Prevention of cell membrane damage
- Modulation of immune responses
- Support of wound healing and antiaging effects
- Antioxidant action strongly complements antimicrobial and anti-inflammatory pathways.
- Antimalarial Activity

Neem has been traditionally used in Africa and Asia as a remedy for malaria. Limonoids such as gedunin and nimbolide inhibit the growth of Plasmodium parasites by disrupting mitochondrial function and protein synthesis (Khalid et al., 2015).

Neem extracts also reduce fever by modulating prostaglandin synthesis and inhibiting the hypothalamic responses triggered during pyrexia.

- Antidiabetic and Metabolic Effects

Neem leaf extracts improve glucose metabolism and insulin sensitivity. Flavonoids, glycosides, and terpenoids enhance pancreatic β-cell function and reduce glucose absorption in the intestine (Chattopadhyay, 1999).

They also reduces oxidative stress in diabetic tissues, protecting against complications.

- Hepatoprotective and Detoxifying Activity

Neem leaves protect the liver from toxin-induced damage. Antioxidant terpenoids and flavonoids stabilize hepatic cell membranes, enhance glutathione levels, and reduce lipid peroxidation (Subapriya & Nagini, 2005).

This supports the traditional use of neem in treating jaundice, digestive issues, and liver inflammation.

- Wound Healing Activity

Neem promotes wound healing by combining antimicrobial, anti-inflammatory, and antioxidant mechanisms. Flavonoids accelerate fibroblast proliferation, collagen deposition, and epithelialization, while terpenoids prevent infection and oxidative damage (Biswas et al., 2002).

Figure 2.2: Image of Azadirachta indica leaves.

Source: Biswas et.al.(2002)

2.4. Phytochemical Properties of *Azadirachta indica*

Azadirachta indica (neem) leaves are rich in diverse classes of secondary metabolites that are responsible for the plant's wide range of pharmacological activities. These phytochemicals include flavonoids, terpenoids, cardiac glycosides, tannins, saponins, steroids, limonoids, and alkaloids, many of which contribute to the antimicrobial, anti-inflammatory, antioxidant, antimalarial, and immunomodulatory effects associated with the plant (Biswas et al., 2002; Subapriya and Nagini, 2005).

2.4.1. Flavonoids

Flavonoids are among the most abundant phytochemicals in neem leaves. Compounds such as quercetin and catechin possess strong antioxidant and anti-inflammatory properties. They scavenge reactive oxygen species (ROS), reduce lipid peroxidation, inhibit inflammatory enzymes, and disrupt microbial enzymatic processes (Panche et al., 2016). Quantitatively, neem leaves often show high flavonoid concentrations, supporting their strong antioxidant capacity.

2.4.2 Terpenoids

Neem is particularly known for its terpenoid-rich profile. Terpenoids such as azadirachtin, nimbolide, and nimbin exhibit potent antimicrobial and cytotoxic properties. They destabilize microbial membranes, inhibit biofilm formation, and interfere with signaling pathways associated with inflammation and oxidative stress (Rauf et al., 2018). Terpenoids are also important contributors to antioxidant activity.

2.4.3 Cardiac Glycosides

Cardiac glycosides present in neem leaves, although traditionally associated with cardiovascular activity, also play roles in antimicrobial defense. They inhibit Na^+/K^+-ATPase

in microbial cells, altering ion balance and reducing viability. Their presence in neem contributes to the plant's broad-spectrum therapeutic effects (Venturini et al., 2013).

2.4.4 Tannins

Tannins in neem leaves contribute to antimicrobial, antioxidant, and astringent properties. They work by precipitating proteins in microbial cell walls, inhibiting enzymes, and neutralizing free radicals. Although present in lower concentrations compared to flavonoids, tannins enhance the plant's overall pharmacological effects (Nwankwo et al., 2020).

2.4.5 Steroids

Plant steroids present in neem leaves have anti-inflammatory and antimicrobial activities. They modulate inflammatory signaling pathways, particularly COX-2, thereby reducing the production of pro-inflammatory mediators (Subapriya & Nagini, 2005). Steroids also contribute to membrane stabilization.

2.5. Antioxidant Properties of Azadirachta indica

Antioxidants are molecules capable of neutralizing reactive species, thereby protecting biomolecules from damage and maintaining cellular redox homeostasis. In neem, antioxidant activity is primarily mediated by flavonoids, terpenoids, and tannins.

Azadirachta indica leaves possess strong antioxidant properties attributed to their rich content of flavonoids, terpenoids, tannins, and other secondary metabolites. These compounds work through multiple biochemical mechanisms to neutralize reactive species and protect biological systems from oxidative damage (Subapriya and Nagini, 2005; Biswas et al., 2002). Neem leaf extracts, especially methanolic extracts have been widely reported to exhibit significant free-radical scavenging potential. In-vitro antioxidant assays such as DPPH, and FRAP consistently show that neem leaves reduce radical species in a concentration-dependent manner (Nwankwo et al., 2020; Kharwar et al., 2020). This activity is primarily

attributed to the high levels of flavonoids, which contain hydroxyl and conjugated aromatic structures capable of donating hydrogen atoms or electrons to stabilize reactive molecules (Panche et al., 2016).

1. **Mechanism of Action:**

- Free Radical Scavenging: Flavonoids donate hydrogen atoms to free radicals, forming stable, non-reactive molecules.
- Metal Chelation: Polyphenolic structures bind transition metals (Fe^{2+}, Cu^{2+}), preventing Fenton-type reactions that generate hydroxyl radicals.
- Reduction of Oxidative Enzymes: Compounds in neem can modulate enzyme activities (e.g., NADPH oxidase), decreasing radical formation.

2. **Biochemical Relevance:**

- Antioxidants stabilize reactive intermediates, thereby preventing lipid peroxidation, protein oxidation, and DNA damage.
- By reducing oxidative stress in pathogens, antioxidants enhance the antimicrobial efficacy of phytochemicals.
- Antioxidant activity contributes indirectly to anti-inflammatory effects by limiting ROS-mediated activation of inflammatory pathways such as MAPK (Subapriya and Nagini, 2005).

3. **Empirical Evidence**:

Methanolic extracts of neem leaves have demonstrated strong in-vitro antioxidant activity, often measured using DPPH, FRAP, or ABTS assays. The activity correlates strongly with flavonoid and terpenoid content, suggesting these compounds are the primary contributors to radical scavenging capacity (Nwankwo et al., 2020; Rauf et al., 2018).

2.6. Antimicrobial Activity of Azadirachta indica

Azadirachta indica has been extensively recognized for its broad-spectrum antimicrobial potential against bacteria, fungi, viruses, and parasites. This activity is largely attributed to its diverse array of bioactive phytochemicals, including azadirachtin, nimbin, salannin, tannins, and steroids, which act synergistically to disrupt microbial growth and survival (Biswas et al., 2002).

2.6.1. Antibacterial Activity

Neem exhibits significant antibacterial activity against both Gram-positive and Gram-negative bacteria. Studies have demonstrated strong inhibitory effects against:

- Staphylococcus aureus,
- Escherichia coli
- Salmonella typhi
- Pseudomonas aeruginosa
- Streptococcus pyogenes
- Klebsiella pneumoniae and Bacillus subtilis (Subapriya and Nagini, 2005).

The antibacterial mechanism has been linked to:

- Disruption of bacterial cell walls and membranes by limonoids and tannins, leading to leakage of intracellular contents (Chakraborty et al., 2015).
- Inhibition of key bacterial enzymes, which interferes with metabolic pathways.
- DNA and protein denaturation due to the presence of phenolic compounds and flavonoids.
- Interference with quorum sensing, thereby reducing bacterial virulence and biofilm formation (Okemo et al., 2001).

Neem leaf extracts, particularly ethanolic and methanolic extracts, have shown higher antibacterial potency than aqueous extracts, suggesting that active compounds are better extracted in organic solvents (Sarkar et al., 2010).

2.6.2. Antifungal Activity

Neem also demonstrates potent antifungal effects. Extracts of A. indica have shown inhibitory activity against species such as:

- Candida albicans
- Aspergillus niger
- Aspergillus flavus
- Microsporum gypseum
- Trichophyton rubrum. (Alzohairy, 2016; Chattopadhyay, 1996).

The antifungal mechanisms include:

- Blocking ergosterol synthesis, destabilizing fungal cell membranes.
- Inhibition of spore germination.
- Interference with fungal respiratory enzymes due to limonoids and flavonoids (Khalil, 2013).

2.6.3. Antiviral Activity

Though less studied, neem extracts have shown activity against several viruses, including:

- HIV (inhibiting replication and cell fusion)
- Herpes simplex virus (HSV)
- Vaccinia virus. (Girish & Bhat, 2008).

The antiviral effects are mainly due to polysaccharides, sulphurous compounds, and flavonoids which prevent viral adhesion, replication, and protein synthesis (Barua & Chakraborty, 2013).

2.6.4. Antiparasitic (Antimalarial and Anthelmintic) Activity

Neem's antiparasitic activity is well-documented. The compound gedunin exhibits strong antimalarial activity by inhibiting the growth of Plasmodium falciparum, disrupting protein processing and parasite development (Udeinya et al., 2004).

Neem leaf extracts also show anthelmintic activity against intestinal worms by:

- Inducing neuromuscular paralysis
- Causing death of parasites by tannin-mediated protein binding. (Ibrahim et al., 2013).

2.6.5. Biochemical Basis of Neem's Antimicrobial Properties

From a biochemistry perspective, the antimicrobial activity of *Azadirachta indica* is rooted in the ability of its bioactive constituents to:

- Act as redox-active compounds, altering microbial oxidative balance.
- Form complexes with bacterial proteins, disrupting enzymatic activities.
- Insert into lipid bilayers, increasing membrane permeability.
- Act synergistically, multiple phytochemicals produce a stronger combined effect than individual compounds.

Thus, neem's antimicrobial activity arises from a multifaceted biochemical assault, making it effective against a wide range of pathogens and reducing the risk of resistance development.

2.7. Anti-Inflammatory Activity of Azadirachta indica

Azadirachta indica (Neem/Dongoyaro) possesses potent anti-inflammatory properties that have been well documented in experimental, biochemical, and clinical studies. Its anti-inflammatory effects are mediated through multiple molecular pathways involving cytokine regulation, inhibition of inflammatory enzymes, antioxidant modulation, and stabilization of cellular membranes (Subapriya & Nagini, 2005).

2.7.1. Inhibition of Pro-Inflammatory Enzymes

One of the major biochemical mechanisms involved in neem's anti-inflammatory action is the inhibition of cyclooxygenase (COX-1 and COX-2) and lipoxygenase (LOX) enzymes, which are responsible for the synthesis of inflammatory mediators such as prostaglandins and leukotrienes (Kaur et al., 2004).

Neem extracts especially nimbidin, nimbin and sodium nimbidate, demonstrate reduction in:

- Prostaglandin E_2 (PGE_2) levels
- Leukotriene B_4 (LTB_4) production
- Edema formation. (Sengupta et al., 2012).

This shows that neem acts similarly to non-steroidal anti-inflammatory drugs (NSAIDs), but without the gastric toxicity associated with synthetic NSAIDs.

2.7.2. Modulation of Cytokines and Immune Response

Neem's anti-inflammatory effect is also mediated through the downregulation of pro-inflammatory cytokines. Several studies have shown that neem leaf extracts reduce:

- Tumor necrosis factor-alpha (TNF-α)
- Interleukin-1β (IL-1β)
- Interleukin-6 (IL-6)
- Interferon-γ (IFN-γ). (Girish & Bhat, 2008).

This immunomodulatory action results in the suppression of chronic inflammation, making neem useful in inflammatory diseases such as arthritis and dermatitis.

2.7.3. Membrane-Stabilizing and Anti-Edematous Properties

Nimbidin, a major bioactive compound in neem, has been shown to stabilize lysosomal membranes, thereby preventing the release of hydrolytic enzymes that contribute to tissue damage during inflammation (Chattopadhyay, 1998).

In animal models of inflammation (carrageenan-induced paw edema and cotton pellet-induced granuloma), neem extracts significantly reduced swelling and inflammation (Chattopadhyay and Banerjee, 1997).

This demonstrates neem's potent anti-edematous effect.

2.7.4. Reduction of Oxidative Stress–Induced Inflammation

Neem indirectly reduces inflammation by enhancing endogenous antioxidant defenses. Neem's flavonoids and limonoids; scavenge reactive oxygen species (ROS), prevent lipid peroxidation in inflamed tissues and reduce oxidative modifications of proteins and DNA (Biswas et al., 2002).

This oxidative protection contributes to the overall reduction of inflammatory processes.

2.7.5. Inhibition of Nitric Oxide (NO) and Reactive Nitrogen Species (RNS)

Overproduction of nitric oxide by inducible nitric oxide synthase (iNOS) contributes to chronic inflammation. Neem extracts inhibit: iNOS expression, Nitric oxide production and Peroxynitrite formation (Sarkar et al., 2010).

This reduces tissue injury caused by reactive nitrogen species in inflammatory conditions.

2.7.6. Biochemical Basis of Neems's Anti-Inflammatory Mechanisms

From a biochemical perspective, neem reduces inflammation through:

- Enzyme inhibition: COX, LOX, iNOS
- Cytokine modulation: ↓ IL-1β, IL-6, TNF-α
- Membrane stabilization: Protects lysosomes
- Antioxidant effects: Reduces ROS/RNS
- Prevention of edema and granuloma formation

These multifaceted actions explain neem's traditional use in treating inflammatory diseases such as fever, arthritis, skin infections, and gastrointestinal inflammation.

2.8 Correlation between Phytochemicals and Antioxidant activity in Azadirachta indica

In *Azadirachta indica*, flavonoids and terpenoids are the primary contributors to free-radical scavenging due to their hydroxyl groups and ability to stabilize oxidation reactions (Biswas et al., 2002). Quantifying these phytochemicals and statistically correlating them with antioxidant values helps validate the mechanisms underlying neem's antimicrobial and anti-inflammatory effects.

2.8.1. Flavonoids as Primary Contributors to Antioxidant Capacity

Flavonoids are consistently identified as the strongest contributors to the antioxidant activity of *Azadirachta indica* leaves. Compounds such as quercetin, catechin, rutin, and kaempferol possess multiple hydroxyl groups that readily donate electrons or hydrogen atoms to neutralize reactive oxygen species (ROS) (Panche et al., 2016).

Their antioxidant mechanisms include:

- Direct scavenging of radicals (•OH, $O_2^{\bullet -}$, NO•)
- Metal chelation, reducing Fe^{2+} and Cu^{2+}-induced radical generation
- Inhibition of lipid peroxidation in biological membranes

Neem leaves typically contain high concentrations of flavonoids, and strong positive correlations have been reported between flavonoid content and DPPH/ABTS scavenging activity (Nwankwo et al., 2020).

2.8.2. Terpenoids Contribute to Radical Scavenging

Terpenoids such as nimbin, nimbolide, azadirachtin, and other triterpenes also contribute to antioxidant capacity. Although less reactive than flavonoids, these compounds exhibit:

- Free-radical scavenging
- Inhibition of ROS-producing enzymes, including xanthine oxidase
- Modulation of redox-sensitive signaling pathways (Rauf et al., 2018)

Their lipophilic nature allows them to integrate into cell membranes, preventing oxidative deterioration of phospholipids.

2.8.3. Tannins Add Additional Antioxidant Strength

Tannins present in neem leaves, although in smaller amounts, still contribute to antioxidant activity due to their strong hydrogen-donating ability and capacity to precipitate pro-oxidant proteins. They inhibit lipid peroxidation and stabilize cell membranes (Nwankwo et al., 2020). Studies report moderate correlations between tannin content and antioxidant assays such as FRAP and DPPH.

2.8.4. Cardiac Glycosides and Saponins Provide Minor Antioxidant Effects

Cardiac glycosides primarily exert physiological effects via Na^+/K^+-ATPase inhibition, but several glycosidic structures exhibit weak-to-moderate antioxidant properties, especially through:

- Metal ion chelation
- Inhibition of ROS formation

Saponins also exhibit mild antioxidant activity, often acting synergistically with stronger antioxidants by improving solubility and membrane penetration of other compounds (Sarker and Nahar, 2007).

These compounds generally show low or weak correlations compared to flavonoids and terpenoids.

2.8.5. Steroids Contribute Indirectly Through Anti-Inflammatory Effects

Plant steroids present in neem contribute indirectly to antioxidant activity by:

- Suppressing ROS-inducing inflammatory mediators
- Stabilizing lysosomal membranes
- Preventing oxidative enzyme release

Though their contribution is secondary, they enhance overall antioxidant behavior when present with flavonoids and polyphenols.

2.8.6. Synergistic Interactions Among Phytochemicals

The antioxidant activity of neem leaves is not due to a single compound but arises from synergistic interactions among its phytochemicals. For example:

- Flavonoids + terpenoids → enhanced radical scavenging
- Tannins + flavonoids → stronger metal-chelating capacity

These synergistic interactions explain why crude extracts often show stronger antioxidant effects than isolated compounds.

CHAPTER THREE

MATERIALS AND METHODS

3.1 APPARATUS AND EQUIPMENT

The apparatus and equipment used for this study were obtained from the Chemistry Laboratory of the University of Benin and were confirmed to be in proper working condition prior to use. The equipment included:

- Analytical balance
- UV–Visible spectrophotometer
- Oven dryer
- Mortar and pestle
- Whatman filter paper
- Rotary evaporator
- Conical flasks
- Measuring cylinders and pipettes
- Test tubes and test tube racks
- Funnel
- Hot plate
- Refrigerator
- Beaker (50ml, and 200ml)
- Sieve
- Electronic sensitive weighing balance
- Glass stirrer
- Spatula
- Concentration jars
- Universal bottles

- Incubator
- Gloves
- Face mask
- EDTA bottle
- Volumetric flask
- Water bath
- Stopwatch
- Aluminum foil paper
- Masking tape (For labeling)

3.1.2 Reagents and Chemicals

All reagents used were of analytical grade and were obtained from the Chemistry Laboratory, University of Benin. These included:

- Methanol
- Distilled water
- Ferric chloride solution
- Chloroform
- Acetic anhydride
- DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent for antioxidant assay
- Aluminum chloride
- Iron(III) chloride
- Sodium carbonate
- Hydrochloric acid (HCl)
- Glacial acetic acid
- Tripyridyl Triazine (TPTZ)
- Molybdate

- Sodium hydroxide (NaOH)
- Methylene red indicator
- Nitric oxide
- Ascorbic acid
- Sulphuric acid (H_2SO_4)
- Copper (II) sulphate ($CuSO_4$)
- Potassium sulphate (K_2SO_4)

3.2 Sample Collection and Preparation

3.2.1 Collection of Plant Material

Fresh *Azadirachta indica* (dongoyaro) leaves were obtained from Ring-road market, Benin city, Nigeria. The plants were identified and authenticated by Prof. H. A. Akinnibosun at the Department of Plant Biology and Biotechnology, University of Benin, Benin city, Edo state, Nigeria.

3.2.2 Preparation of Plant Material

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

3.3. Qualitative phytochemical screening procedure

Phytochemicals are bioactive constituents of medicinal plants which are not nutrients but very useful to the plants. Some bioactive constituents of methanolic extract were analysed qualitatively for Flavonoids, Tannins, Cardiac Glycosides, Saponins, Steroids, Terpenoids, Phenols, Phlobatanins, Coumarin, Anthraquinone and Alkaloids. Phytochemical screening

was carried out on the samples after undergoing methanol extraction, using standard procedures to identify the secondary metabolites (Harborne, 1973; Trease and Evans, 1989; Sofowora 1993).

3.3.1. Solvent extract preparation

Pulverised sample (150g) was measured and placed in bottles. 500mL of absolute ethanol was used to soak the samples, subsequently stirred 3 times daily and kept in dark room for 72 hours. Supernatant was filtered using a muslin cloth and the solvent evaporated using a rotary evaporator. The dried extracts were weighed and kept in sterile universal bottles for freezer at a temperature of -4˚C for some time.2g of the crude extract was weighed and used for phytochemical screening.

3.3.2. Test for flavonoids

5mL of 10% ammonia was added to portion of an aqueous filtrate of the extract. Then concentrated sulfuric acid was added. Observed yellow colour indicates the presence of flavonoids.

3.3.3. Test for tannins

1mL of (0.5g/5mL) ethanol extract was boiled in 2ml of water in a test tube and filtered. A 3 drops of 0.1% ferric chloride was added and observed for brownish green to a blue-black colouration.

3.3.4 Test for cardiac glycosides (Keller-Killiani test)

1mL of 0.5g/5ml aqueous extract was treated with glacial acetic acid containing one drop of ferric chloride solution. 1mL of concentrated sulfuric was gently added. A browning at the interface indicated the presence of a deoxysugar characteristic of cardenolides. Hence, the presence of cardiac glycosides.

3.3.5 Test for saponin (Frothing test)

The ability of saponins to produce frothing in aqueous solution was used as a screening test for saponins. 1mL of extract (0.5g/5mL of distilled water) was mixed with 5mL of distilled water and shaken vigorously for a stable persistent froth, indicating the presence of saponin. This was further confirmed by adding 3 drops of olive oil and shaking vigorously after which it was observed for the formation of an emulsion.

3.3.6 Test for steroids

2mL of concentrated acetic anhydride was added to 0.5mL of (0.5g/5mL) ethanol extract of each sample with 2mL concentrated sulfuric acid. The colour changed from violet to blue or green colouration was positive for steroids.

3.3.7 Test for terpenoids (Salkowski test)

1mL of the extract in a test tube was mixed with 2mL of concentrated chloroform and 3ml of concentrated sulfuric acid. Reddish brown coloration at the interface confirmed the presence of terpenoids.

3.3.8 Test for phenols

3 drops of 10% aqueous FeCl3 solution were added in a test tube to 5mL of (0.5g/5mL) ethanol extract. Formation of blue or green coloration indicated the presence of phenols.

3.3.9 Test for phlobatanins

3mL of (0.5g/5mL) ethanol extract was added to 2mL of 1% HCl and the extract was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatanins.

3.3.10 Test for Coumarin:

5mL of (0.5g/5mL) ethanol extract was dissolved in 2mL of hot distilled water and divided into two parts. Half of the volume was a control; the other part 0.5ml of 10% NH4OH was added.

3.3.11 Test for alkaloids:

Mayer's test: 1mL of (0.5g/5mL) ethanol extract was mixed with 3drops of Mayer's reagent. Cream coloured precipitate formation confirmed the presence of alkaloids.

3.3.12 Test for anthraquinone:

5mL of benzene was added to 1mL of (0.5g/5mL) ethanol extracts in a test tube and shaken vigorously in 2.5mL concentrated NH3. Formation of pink-red colouration at the lower phase was indicative of the presence of free Anthraquinone.

3.4. Quantitative determination of phytochemicals

3.4.1. Estimation of alkaloids (Madhu et al., 2016).

To of test extract, 5ml of pH 4.7 phosphate buffer was added and 5ml BCG solution and shake a mixture with 4ml of chloroform. The extracts were collected in a volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 760nm against blank prepared as the above with extract. Atropine was used as a standard and compared the assay with atropine equivalent.

3.4.2. Estimation of Flavonoids (Madhu et al., 2016)

Total flavonoid content was determined by the Aluminum chloride method using Quercetin as a standard.of test sample and 4ml of water were added to a volumetric flask. After 5mins, 0.3ml of 5% Sodium nitrite and 0.3ml of 10% Aluminum chloride were added. After 6mins incubation at room temperature, 2ml of 1M Sodium hydroxide was added to the reaction

mixture. Immediately, the final volume was made up to with distilled H2O. The absorbance of the reaction mixture was read at 510nm against a blank spectrophotometrically.

3.4.3 Estimation of Steroids (Madhu et al., 2016)

of extract of steroid solution was transferred into volumetric flask. Sulphuric acid (4N, 2ml) and Iron (III) chloride (0.5% w/v 2ml) were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5ml). The mixture was heated with occasional shaking and diluted to the mark with diluted water. The absorbance was measured at 780 nm against the reagent blank. Stigmasterol was used as standard.

3.4.4 Estimation of Terpenoids (Alessandra et al., 2020)

To 75ul plant extract, 250ul of vanillin solution (50mg/ml) and 500ul of Sulphuric acid (99.5%). The tube was heated in a water bath (60oC) for 20mins and then transferred into an ice bath followed by the addition of 2500ul of acetic acid (99.5%). The resulting solution was cooled for 20mins and absorbance was measured at 548nm. Beta-sitosterol was used as a standard.

3.4.5 Estimation of Coumarin (Ameen et al., 2021)

A 0.5ml of 5NNaOH was added to the solution of 1 ml of the extract (0.5g in 1 ml methanol). The mixture was heated at 80oC for 5mins. After cooled, 0.75ml of 5N H2SO4 was added and mixed thoroughly, then, 0.25g of anhydrous NaHCO3 was also added and transferred to the extractor and made up to 50 ml with pet-ether for 3hrs. About 20ml of H2O was added to the pet-ether extract and carefully evaporate the pet-ether in water bath at 50-55oC. The aqueous solution was transferred to a volumetric flask and made up to 50ml with mixing. 25ml of aqueous solution was pipetted into a flask and 1%. Na2CO3 solution was added and heated in a water bath at 75% for 15mins and cooled. 5 ml of the diazonium solution was

added and stand 2 hours. The absorbance at 540nm against reagent blank. Esculin was used as standard.

3.4.6 Estimation of Phenols (Tofighi et al., 2016)

The methanol solution of each sample (0.2) was mixed with folin-ciocalteu reagent (2 ml, 1:10 diluted with distilled H2O). After 5mins, saturated NaHCO3 solution (1.5ml, 60g/L distilled water) was added. The mixture were allowed to stand for 90mins at room temperature and absorbance of the solution was measured at 725nm. The same procedure was repeated for different concentrations of gallic acid solution (0.2).

3.4.7 Estimation of Cardiac glycosides (Tofighi et al., 2016)

10% extract was mixed with of freshly prepared Baljet's reagent (95ml of 1% picric of 5ml of 10%NaOH). After an hour, the mixture was diluted with 20ml distilled water and the absorbance was measured at 495nm. Securidaside was used as standard.

3.4.8 Estimation of Tannins (Kritha and Indira, 2016)

Tannins were determined by the folin ciocalteu method.

of sample extract was added to the volumetric flask containing 7.7ml of distilled water. The mixture was shaken well and kept at room temperature for 30mins, a set of reference standard solutions of tannic acid (20, 40, 60, 80) in the same manner as described for sample extract. Absorbance for test and standard solutions were measured against reagent blank at 700nm.

3.5. DETERMINATION OF IN VITRO ANTIOXIDANT ACTIVITY

3.5.1 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 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 (FeCl3.6H2O) solution was added to of the extracts

at concentrations of 100-600uM. The reaction mixtures were incubated at 370c for 30mins and the increase in absorbance at 593nm was measured. FeSO4 was used for the calibration curve and ascorbic acid served as the positive control. FRAP values (expressed as mM.Fe(ɥ/g) of the extract) for the extracts were then extrapolated from the standard curve.

3.5.2 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 (1v) 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) was added to molybdate reagent solution. These tubes were incubated at 950c 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.

3.5.3 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 read at 517nm. All tests were performed in triplicate. Ascorbic acid was used as standard control, with similar concentrations as 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 (%) = [(A0 – A1) / (A0)] x 100,

Where; A0 was the absorbance of DPPH radical + Methanol.

A1 was the absorbance of DPPH radical + sample extract or standard.

The 50% inhibitory concentration value (IC50) was calculated as the effective concentration as the effective concentration of the extract that is required to scavenge 50% of the DPPH free radicals.

3.5.4 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 generated from sodium nitroprusside in aqueous solution at pysiological pH with oxygen to produce nitrite ions which is measured by 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 (p7.4) 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 25oC. after 150 min, 0.5ml of incubated solution was withdrawn and mixed with 0.5ml of Griess reagent[1 sulfanilic acid reagent(0.33% prepared in 20% glacial acetic acid at room temperature for 5min with of napthylethylene diamine dihydrochloride (0.1%w/v)]. the mixture was incubated at room temperature for 30min, followed by the measurement of absorbance read at 540nm.

CHAPTER FOUR

RESULTS

4.1. Qualitative Phytochemical Screening

The methanolic extract of Dongoyaro was screened for the presence of various classes of secondary metabolites. The results of these qualitative tests are presented in Table 4.1. The screening revealed the presence of flavonoids, cardiac glycosides, steroids, phenols, tannins, and terpenoids. Phlobatannins, anthraquinones, saponins, coumarin, and alkaloids were not detected under the experimental conditions used.

Table 4.1: Qualitative Phytochemical Profile of Methanolic Aloe vera Leaf Extract

PHYTOCHEMICALS	**SAMPLE B (DONGOYARO)**
FLAVONOIDS	+++
TANNINS	++
CARDIAC GLYCOSIDES	++
PHLOBATANNINS	-
STEROIDS	+++
TERPENOIDS	+++
ANTHRAQUINONE	-
SAPONINS	-
COUMARIN	-
ALKALOIDS	-
PHENOLS	++

Key: (+) = Present; (++) = Abundantly Present; (-) = Absent.

4.2. Quantitative Phytochemical Analysis

Following the positive results from the qualitative screening, the concentrations of total flavonoids, terpenoids, cardiac glycosides, and steroids in the methanolic extract were quantified using spectrophotometric methods. The results, expressed in ug/mL, are shown in Table 4.2. The analysis indicates that flavonoids and cardiac glycosides are the most abundant of the quantified phytochemicals.

Table 4.2: Quantitative Analysis of Major Phytochemicals in Methanolic Dongoyaro Leaf Extract

PHYTOCHEMICALS	CONCENTRATIONS (ug/mL)
TOTAL FLAVONOIDS	862.21
TOTAL TERPENOIDS	305.48
TOTAL CARDIC GLYCOSIDES	527.75
TOTAL STEROIDS	282.50
TOTAL TANNINS	77.13

Values are the mean of triplicate determinations ± standard deviation.

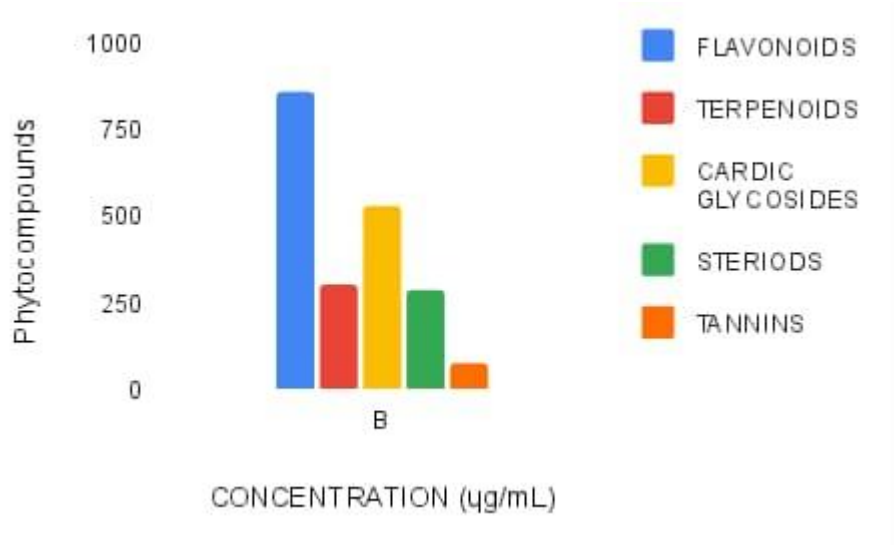

Figure 4.1: A Barchart Showing the quantity of each phytochemical present in the leaves of Azadirachta indica in ug/ml.

4.3: In Vitro Antioxidant Properties

The antioxidant capacity of the Dongoyaro methanolic extract was evaluated using four different assays: DPPH radical scavenging, Ferric Reducing Antioxidant Power (FRAP), Nitric Oxide (NO) scavenging, and Total Antioxidant Capacity (TAC). The activity of the extract was compared against ascorbic acid, a standard antioxidant.

4.3.1. DPPH radical scavenging activity of Dongoyaro extract versus ascorbic acid

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

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

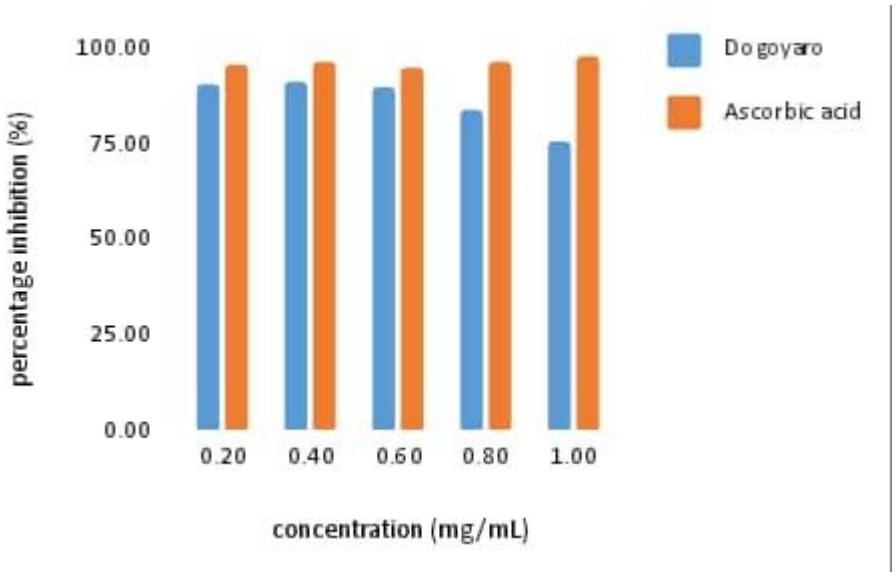

Figure 4.2: A bar chart showing the DPPH radical scavenging activity of Dogoyaro extract versus ascorbic acid.

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

Table 4.4: 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
Dongoyaro	71.28	58.14	52.60	44.18	42.34
Ascorbic acid.	91.62	92.50	93.01	92.48	93.53

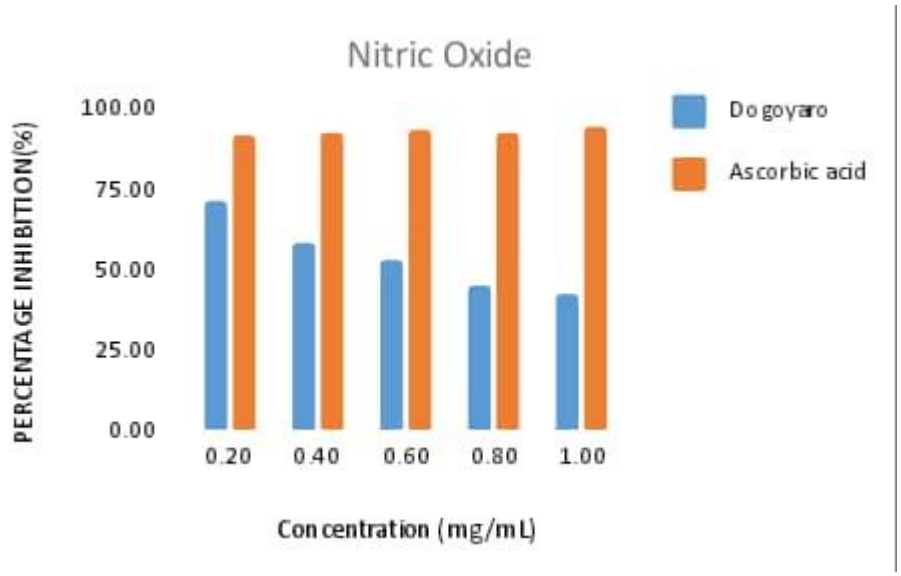

Figure 4.3: A bar chart showing nitric oxide scavenging activity of Dogoyaro extract and ascorbic acid across concentrations.

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

Table 4.5. 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
Dongoyaro	52.45	48.06	46.96	45.72	43.50
Ascorbic acid	90.07	89.76	87.91	89.04	89.04

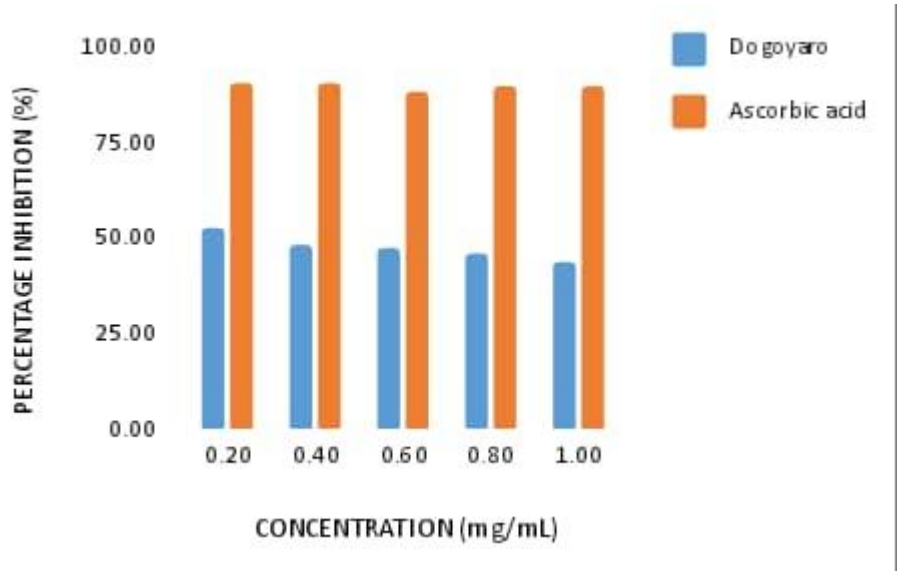

Figure 4.4: A bar chart showing the Ferric reducing antioxidant power (FRAP) of Dogoyaro extract compared with the standard antioxidant.

4.3.4: 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.

Dongoyaro	137.93
Ascorbic acid	194.71

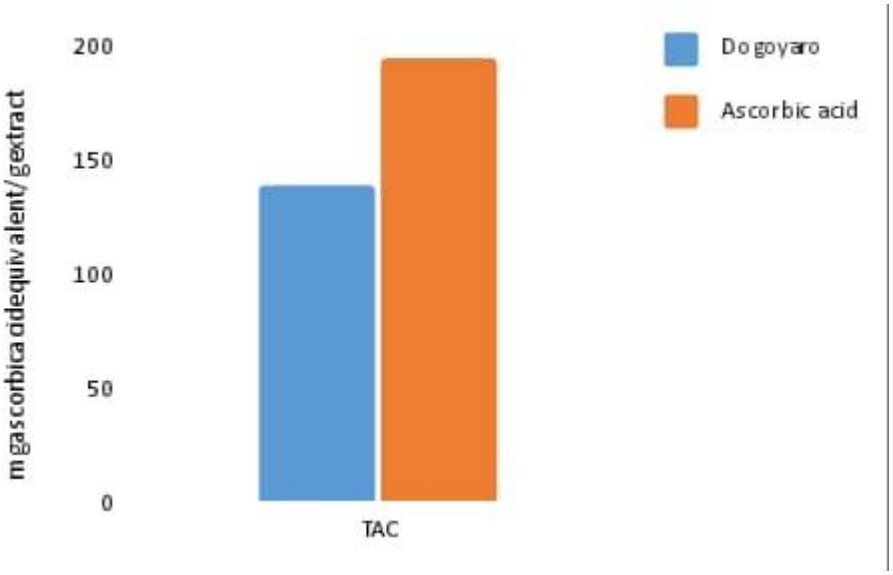

Figure 4.5: A bar chart showing the Total antioxidant capacity of Dongoyaro extract compared with ascorbic acid

CHAPTER FIVE

DISCUSSION

5.1 Discussion of antioxidant activity.

5.1.1 Nitric oxide scavenging activity

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. Although the extract is less potent than the standard, its Nitric oxide (NO) scavenging indicates potential for anti-inflammatory effects, as NO is a known mediator of inflammation and tissue damage.

5.1.2. Ferric Reducing Antioxidant Power

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.

5.1.3 DPPH Radical Scavenging Activity.

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.

5.1.4. Total Antioxidant Capacity.

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 Dongoyaro 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: Dongoyaro 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. Interpretation of Phytochemical Composition

Qualitative and quantitative phytochemical screening revealed the presence of major secondary metabolites including flavonoids, tannins, alkaloids, saponins, terpenoids and cardiac glycosides. These phytochemicals are widely reported to be responsible for the biological activities associated with *Azadirachta indica* (Biswas et al., 2002; Subapriya and Nagini, 2005).

- Flavonoids are potent antioxidants known to scavenge free radicals and suppress oxidative stress–mediated inflammation.
- Tannins possess antimicrobial and antioxidant activities due to their ability to precipitate proteins and inhibit bacterial growth.
- Alkaloids and terpenoids are known to exhibit anti-inflammatory and antimicrobial effects through mechanisms involving enzyme inhibition and membrane disruption.
- Saponins contribute to antimicrobial activity by causing lysis of microbial cell membranes.

The presence of these phytochemicals strongly supports the traditional and scientific use of A. indica in treating microbial infections and inflammatory conditions.

5.3. Correlation Between Phytochemicals and Antioxidant Activity

- Flavonoids contribute significantly to DPPH and TAC values due to their hydrogen-donating nature.
- Tannins and phenolics influence FRAP and NO scavenging activity through electron transfer and metal-chelation mechanisms.
- Terpenoids and alkaloids contribute modestly to radical scavenging but strongly support anti-inflammatory potential.

The decreasing antioxidant activity at higher extract concentrations may reflect interference among different bioactive compounds, a known phenomenon in crude plant extracts (Cowan, 1999).

Overall, the antioxidant results support the phytochemical composition and validate the plant's traditional use for detoxification, inflammation reduction, and protection against oxidative stress.

5.4. Implications for Antimicrobial and Anti-Inflammatory Activity

The phytochemicals identified in *Azadirachta indica* have well-documented antimicrobial and anti-inflammatory properties:

Antimicrobial Implications: Tannins destabilize microbial cell walls and inhibit bacterial enzymes. Saponins disrupt membrane integrity..Alkaloids and terpenoids interfere with microbial DNA and protein synthesis.

These compounds, combined with the extract's significant antioxidant property, suggest that Dongoyaro leaves may effectively control microbial infections. Previous studies have reported potent antimicrobial activity of neem against Staphylococcus aureus, E. coli, Candida albicans, and others (Banso & Adeyemo, 2007).

Anti-Inflammatory Implications: The extract's ability to scavenge NO radicals — key mediators of inflammation — and its possession of phytochemicals that modulate COX, LOX, and NF-κB pathways support its anti-inflammatory potential. Flavonoids and terpenoids, abundant in the extract, are known inhibitors of inflammatory cytokines (Subapriya & Nagini, 2005).

Thus, even though this study did not directly assess antimicrobial or anti-inflammatory properties, the phytochemical and antioxidant results provide strong scientific evidence that A. indica leaves possess these therapeutic potentials.

5.5 CONCLUSION

This study concludes that Azadirachta indica leaves contain rich phytochemical constituents that contribute substantially to their antioxidant properties. Although the antioxidant activity of the methanolic extract was lower than that of ascorbic acid, the extract demonstrated significant capacity to scavenge radicals, reduce ferric ions, and indicate strong total antioxidant potential. The findings strongly support the traditional use of Dongoyaro leaves and provide scientific evidence for their possible antimicrobial and anti-inflammatory applications. The phytochemicals identified are consistent with those responsible for these bioactivities in previous studies.

5.6 RECOMMENDATIONS

- Further Studies Using Bioassays: Direct antimicrobial and anti-inflammatory laboratory tests should be conducted to confirm the extract's efficacy against specific pathogens and inflammatory mediators.
- Fractionation of Extracts: Isolating individual phytochemicals may improve antioxidant potency and help identify the most active components.
- In vivo Studies: Animal or clinical trials should be conducted to investigate the safety, toxicity, and pharmacological effectiveness of the extract.
- Standardization of Extracts: Standard doses should be established to optimize therapeutic applications and minimize variations in potency.
- Formulation Studies: The extract may be incorporated into herbal medicines, ointments, or nutraceuticals after appropriate testing.

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