EVALUATION OF ELEMENTAL ANALYSIS, TOTAL ALKALOIDS, ABTS, AMINO ACIDS PROFILE, HEAT INDUCED HAEMOLYSIS AND ANTI-DIABETIC POTENTIAL OF MORINDA CITRIFOLIA

BY

KEHINDE SAMUEL OSAFEHINTI

PSC2008007

DEPARTMENT OF CHEMISTRY

FACULTY OF PHYSICAL SCIENCE

UNIVERSITY OF BENIN

FEBRUARY, 2025.

EVALUATION OF ELEMENTAL ANALYSIS, TOTAL ALKALOIDS, ABTS, AMINO ACIDS PROFILE, HEAT INDUCED HAEMOLYSIS AND ANTI-DIABETIC POTENTIAL OF MORINDA CITRIFOLIA

BY

KEHINDE SAMUEL OSAFEHINTI

PSC2008007

A PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONS) IN CHEMISTRY (BSc. CHEMISTRY)

FEBRUARY, 2025

CERTIFICATION

This is to certify that this research project was carried out by **KEHINDE SAMUEL OSAFEHINTI** with the matriculation number **PSC2008007** under the supervision of **Dr. O.K. OGBEIDE**

____________________________	____________________________
KEHINDE SAMUEL OSAFEHINTI	**DATE**
(STUDENT)	

____________________________	____________________________
DR. O.K. OGBEIDE	**DATE**
(PROJECT SUPERVISOR)	

____________________________	____________________________
PROF. E.E.I. IRABOR	**DATE**
(H.O.D)	

DEDICATION

I humbly dedicate this research project to God Almighty, whose grace, wisdom, and guidance has made this endeavor a success and guided me through His strength at the esteemed University of Benin.

In addition, I also dedicate this project to my parents, **Mr Emmanuel Osafehinti and Late Mrs. Kemisola Osafehinti**, whose unwavering support, encouragement, and sacrifices have been my greatest source of inspiration. Your guidance and belief in my dreams have been the foundation of my academic journey. I am forever grateful for your love, wisdom, and prayers, which have carried me through every step of this process.

Thank you for everything and may God continue to bless you both.

ACKNOWLEDGEMENT

First and foremost, I am deeply grateful to Almighty God for His grace, guidance, and provision throughout the journey of completing this final year project.

I also wish to express my heartfelt appreciation to my beloved parents, **Pastor Emmanuel Osafehinti and Late, Mrs. Kemisola Osafehinti**, for their unwavering love, sacrifices, and support. Your prayers and encouragement have been my greatest source of strength and motivation.

My sincere gratitude to my siblings for their constant support, understanding and encouragement. Your belief in me has been a source of inspiration, and I am truly grateful for your presence in my life.

To my supervisor, **Dr O.K. Ogbeide**, I am profoundly thankful for your exceptional guidance, constructive feedback, and patience throughout this project. Your expertise and support have been invaluable, and I deeply appreciate the time and effort you dedicated to my work.

Finally, I would like to thank my lecturers, colleagues most especially Joseph and the University of Benin for the knowledge, resources, and opportunities provided to make this project a success. To everyone who contributed directly or indirectly to the completion of this work, you have my lifelong gratitude.

TABLE OF CONTENT

CHAPTER TWO

ABSTRACT

This study investigates the medicinal properties of the leaf extract of *Morinda Citrifolia*, commonly known as Noni, with a particular focus on its anti-diabetic potential, antioxidant activity, and biochemical composition. The research encompasses an in-depth analysis of the *Morinda Citrifolia* extract phytochemical composition, antidiabetic activity, elemental composition, heat-induced hemoglobin inhibition, and alkaloid and amino acid profiles. After identifying bioactive components by phytochemical screening, the extract was tested in vitro for its potential as an antidiabetic and its capacity to prevent hemoglobin denaturation. In addition to analyzing the alkaloid and amino acid profiles, elemental analysis was used to ascertain the concentration of both beneficial and detrimental components. An inhibitory assay was used to measure the antidiabetic action, and absorbance measurements were used to identify heat-induced hemoglobin inhibition.

The extract contained alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids and phenolic chemicals, according to phytochemical screening. With a maximal inhibitory activity of 49.10% at a dosage of 0.5 mg/mL, the antidiabetic activity demonstrated a dose-dependent rise in inhibition. Significant action was shown by the heat-induced hemoglobin inhibition, which peaked at 84.07% at 300 mg/mL. Essential elements including calcium, magnesium, zinc, and potassium were found by elemental analysis; dangerous elements like lead and chromium were not found in any discernible amounts. The total protein or amino acid content in the extract was 111.58840, and there was 0.2 g of total alkaloids.

Morinda Citrifolia extract has significant biological properties, such as antioxidant and antidiabetic actions, as well as the capacity to prevent hemoglobin denaturation. The extract's possible therapeutic applications are further supported by the presence of vital components and bioactive phytochemicals. The presence of protein and amino acids, however, raises the plant's nutritional and medicinal value. These results lay the groundwork for additional research into *Morinda Citrifolia's* active ingredients and potential medical uses.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Medicinal herbs are plants that, either in their natural state or processed into extracts, supplements, or medications, are used for their therapeutic qualities. Ancient Egyptian, Chinese, Indian, and Greek civilizations were among the first to use medicinal plants. These plants served as the foundation for traditional medical systems including Ayurveda, Traditional Chinese Medicine (TCM), and Unani medicine (Bisset & Wichtl, 2001).

In both conventional and contemporary healthcare systems, medicinal herbs are essential. Alkaloids, flavonoids, tannins, and essential oils are among the bioactive substances found in these plants that support their therapeutic qualities. The identification of anti-inflammatory, antioxidant, antibacterial, anti-mutagenic, anti-carcinogenic, and antimalarial medications has considerable promise thanks to medicinal plants utilized in traditional medicine (Ogbeide *et al*., 2020).

Medicinal herbs are being intensively studied for their possible advantages as a result of growing resistance to synthetic medications and a growing desire for natural therapies. The global market for herbal medicines continues to expand, highlighting the significance of these plants in disease prevention and treatment. Plant extracts continue to be the most abundant source of novel medications, despite the fact that millions of chemical compounds with therapeutic potential are being created in laboratories from time to time (Ogbeide *et al*., 2018).

Medicinal plants have played an essential role in human health for thousands of years, serving as a primary source of treatment for various ailments. Before the development of modern pharmaceuticals, different civilizations depended on plants for their healing properties, extracting beneficial compounds to address health concerns (Farnsworth et al., 1985). These plants are deeply embedded in traditional medicinal systems, including Ayurveda, Traditional Chinese Medicine (TCM), Unani, and African herbal medicine, each offering a unique approach to healing through natural substances (WHO, 2013).

The transmission of knowledge about medicinal plants has historically relied on oral traditions, ancient manuscripts, and, more recently, scientific studies. Plants contain diverse bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and essential oils, which have significant pharmacological effects (Newman & Cragg, 2016). These natural compounds exhibit properties such as anti-inflammatory, analgesic, antimicrobial, and antioxidant effects. Many contemporary drugs have their origins in traditional plant-based treatments. For instance, aspirin was derived from salicylic acid found in willow bark, while quinine, a well-known antimalarial drug, was extracted from the Cinchona tree (Rates, 2001).

Modern scientific research has reinforced the efficacy of many medicinal plants. Advances in fields like phytochemistry and molecular biology have facilitated a deeper understanding of the mechanisms by which these plants exert their therapeutic effects (Gurib-Fakim, 2006). Studies have demonstrated the benefits of curcumin, the active ingredient in turmeric, due to its potent anti-inflammatory and antioxidant properties (Aggarwal & Sung, 2009). Similarly, garlic, which contains allicin, has been shown to promote cardiovascular health by reducing blood pressure and cholesterol levels (Rahman, 2007).

Despite the growing recognition of medicinal plants in modern medicine, challenges related to their standardization and regulation persist. Unlike synthetic pharmaceuticals that have precise chemical compositions, plant-based remedies may vary in their active ingredients due to environmental factors such as soil conditions, climate, and harvesting methods (WHO, 2018). This variability underscores the need for stringent quality control measures to ensure consistency and safety. The World Health Organization (WHO) has developed guidelines for cultivating, processing, and utilizing medicinal plants in healthcare (WHO, 2003).

Another significant challenge facing medicinal plants is their conservation. The increasing global demand for herbal medicines has led to overharvesting and habitat destruction, endangering several plant species (Hamilton, 2004). The loss of these plants not only impacts biodiversity but also threatens the cultural heritage of communities reliant on traditional medicine. Sustainable practices, including controlled cultivation and responsible harvesting, are critical in preserving these valuable resources. Efforts to document and safeguard indigenous knowledge are equally essential to maintaining traditional medicinal practices (Cunningham, 1993).

Medicinal plants have also gained prominence in integrative medicine, where they complement conventional treatments. Many individuals opt for herbal medicine as an alternative or adjunct therapy, particularly for managing chronic diseases such as diabetes, hypertension, and arthritis (Eisenberg et al., 1998). The increasing popularity of holistic health approaches has further fueled interest in plant-based remedies, leading to their incorporation into teas, tinctures, capsules, and topical applications to address a wide range of health concerns (Tilburt & Kaptchuk, 2008).

While medicinal plants offer numerous health benefits, their use requires caution. Self-medication and excessive consumption of herbal remedies can result in adverse reactions, particularly when combined with prescription drugs (Ernst, 2000). Some plants contain potent compounds that may interact with pharmaceuticals, causing unintended side effects. For example, St. John's Wort, commonly used for depression, has been found to interfere with the metabolism of certain medications, diminishing their efficacy (Izzo & Ernst, 2001). Consulting healthcare professionals before using herbal medicine is advisable, particularly for individuals with preexisting medical conditions or those on multiple medications.

Ethnobotanical research has been instrumental in uncovering the therapeutic potential of various plant species. Indigenous knowledge has frequently provided the foundation for scientific inquiry, leading to groundbreaking discoveries in medicine (Fabricant & Farnsworth, 2001). The rosy periwinkle (*Catharanthus roseus*), traditionally used in folk medicine, served as the basis for the development of vincristine and vinblastine, two alkaloids now used in cancer treatment (Cragg & Newman, 2005). This interplay between traditional wisdom and modern science highlights the importance of preserving and respecting indigenous healing practices.

The pharmaceutical industry continues to explore medicinal plants as sources of novel therapeutic agents. Many antibiotics, anticancer drugs, and pain relievers have originated from natural products derived from plants (Butler, 2004). Advances in biotechnology and genetic engineering have expanded research opportunities, facilitating the synthesis and enhancement of plant-derived compounds for medical use. Additionally, medicinal plants contribute to other sectors, including veterinary medicine, agriculture, and cosmetics. The skincare industry, for instance, incorporates botanical extracts in various products due to their healing and anti-aging properties (Baumann, 2007).

With the increasing interest in herbal medicine, universities and research institutions have invested in studying medicinal plants and their applications. Botanical gardens, herbariums, and pharmacognosy departments play a crucial role in preserving and advancing knowledge in this field. Governments and regulatory bodies recognize the necessity of policies that ensure the safe and effective use of herbal medicine (WHO, 2002). In some nations, herbal medicines are integrated into national healthcare systems, promoting a more comprehensive approach to patient care (Bodeker et al., 2005).

The growing preference for medicinal plants reflects a broader societal shift toward natural and preventive healthcare. As people become more aware of the benefits and limitations of synthetic drugs, there is renewed interest in plant-based remedies. The fusion of traditional knowledge with modern scientific research holds great promise for healthcare advancements. However, responsible usage, ethical sourcing, and continued research are crucial for fully unlocking the potential of medicinal plants while ensuring their sustainable use for future generations (Heinrich et al., 2004).

Medicinal plants remain an indispensable part of global healthcare traditions, providing relief and healing for centuries. They exemplify the intricate connection between humans and nature, offering invaluable resources for health and wellness. Whether utilized in traditional medicine, modern pharmaceuticals, or wellness practices, these plants continue to shape approaches to health and disease. Ongoing scientific research will further elucidate their mechanisms of action, ensuring that medicinal plants remain a cornerstone of healthcare, bridging ancient wisdom with contemporary medical advancements.

Research on therapeutic herbs is developing as natural medicine gains popularity. Their medicinal potential can be increased by combining traditional knowledge with contemporary scientific methods. Their continued use in healthcare will be guaranteed by sustainable cultivation, appropriate standardization, and scientific confirmation. It is impossible to undervalue the contribution that medicinal plants provide to medication development, global health, and preventative healthcare.

1.1.1 BACKGROUND OF STUDY

For centuries, medicinal plants have served as vital sources of bioactive compounds, playing a significant role in both traditional and modern medicine. *Morinda Citrifolia* (commonly referred to as Noni) has attracted increasing scientific interest due to its wide-ranging pharmacological benefits, including antioxidant, antimicrobial, anti-inflammatory, and antidiabetic properties. These therapeutic effects are largely linked to the plant's rich phytochemical profile, which includes alkaloids, flavonoids, phenolics, and essential nutrients.

The elemental composition of medicinal plants is crucial in understanding their biological functions, as trace elements contribute to enzymatic activity, metabolic regulation, and antioxidant defense mechanisms. Alkaloids, in particular, are known for their diverse pharmacological effects, including potential roles in blood sugar regulation and anti-inflammatory activities. Antioxidants found in plant extracts help counteract oxidative stress, which is a major contributor to chronic diseases such as diabetes, cardiovascular disorders, and neurodegenerative conditions.

The ability of plant extracts to stabilize red blood cell membranes under thermal stress is an essential factor in evaluating their potential to mitigate inflammation and oxidative damage. Additionally, analyzing the amino acid composition of *M. citrifolia* leaves provides insights into their nutritional and therapeutic significance, as amino acids are essential for metabolic processes, immune function, and protein synthesis.

Given the growing demand for scientifically validated herbal remedies, this study seeks to examine the elemental profile, alkaloid content, antioxidant activity, amino acid composition, heat-induced haemolysis effects, and antidiabetic potential of *M. citrifolia* leaf extract. The findings from this research will contribute to a deeper understanding of the plant's medicinal value and support its application in disease prevention and management.

1.1.1.1 DEFINITION OF TERMS

• Anti-diabetic: Describes drugs that aid in controlling blood sugar levels.
• Heat-induced hemolysis: When high temperatures cause red blood cells to break down.
• ABTS Assay: A technique for assessing a compound's antioxidant potential.
• Total Alkaloid Content: A plant extract's total alkaloid content.
The method of ascertaining a substance's mineral composition is known as elemental analysis.
• Amino Acid Profile: A thorough breakdown of the amino acids that are necessary for the creation of proteins and other metabolic functions.

Morinda Citrifolia leaves are a promising option for medicinal uses due to their rich phytochemical makeup, which includes antioxidants, alkaloids, and vital minerals. The current study intends to investigate the medicinal relevance and biochemical features of *Morinda Citrifolia* leaf extract by assessing its anti-diabetic potential, heat-induced hemolysis inhibition, ABTS radical scavenging activity, total alkaloid content, elemental composition, and amino acid profile.

1.1.1.2 ANTI-DIABETIC STUDIES ON *MORINDA CITRIFOLIA*

Chronic hyperglycemia brought on by insulin resistance, insulin insufficiency, or both is a hallmark of diabetes mellitus (American Diabetes Association, 2021). Millions of individuals worldwide are impacted by this serious global health issue. The International Diabetes Federation (IDF) estimates that 537 million persons worldwide had diabetes in 2021; by 2030, that number is expected to increase to 643 million, and by 2045, it will reach 783 million (IDF, 2021). Diabetes is a major source of morbidity and mortality due to its consequences, which include cardiovascular illnesses, retinopathy, neuropathy, and nephropathy (Forbes & Cooper, 2013).

Insulin therapy and oral hypoglycemic medications (such as metformin and sulfonylureas) are examples of conventional anti-diabetic treatments with drawbacks, such as side effects, exorbitant prices, and a gradual decline in effectiveness (Bailey & Day, 2004). Herbal remedies are therefore becoming more and more popular as alternative or complementary therapies. Because of their capacity to improve insulin sensitivity, regulate blood glucose levels, and offer antioxidant protection against oxidative stress brought on by diabetes, plants such as M. citrifolia have drawn attention (Nayak *et al*., 2011).

According to studies, *Morinda Citrifolia* has hypoglycemic effects by enhancing glucose absorption, inhibiting α-glucosidase, and protecting pancreatic β-cells (Kamiya *et al*., 2008). However, nothing is known about the precise function of *Morinda Citrifolia* leaves in the treatment of diabetes, especially when it comes to their amino acid profile, elemental makeup, and alkaloid content.

Among the many plants studied, *Morinda Citrifolia* (Noni) has gained attention due to its long history in traditional medicine for treating metabolic disorders, including diabetes. The plant contains a rich profile of bioactive compounds such as alkaloids, flavonoids, polyphenols, and iridoids, which have demonstrated hypoglycemic effects in preclinical studies (Deng et al., 2019). These compounds are believed to exert their antidiabetic effects through various mechanisms, including enhancing insulin sensitivity, inhibiting carbohydrate-digesting enzymes (α-amylase and α-glucosidase), and promoting glucose uptake in peripheral tissues (Huang et al., 2022).

Oxidative stress is a key factor in the progression of diabetes, contributing to pancreatic β-cell dysfunction and insulin resistance (Singh et al., 2021). *Morinda Citrifolia* has been reported to possess strong antioxidant properties, which may help mitigate oxidative damage and improve glucose metabolism (Rahmat et al., 2020). Additionally, the plant's anti-inflammatory properties could further support pancreatic function and insulin regulation, reducing the overall burden of diabetes-related complications (Kumar et al., 2018)..

This study seeks to contribute to the growing body of knowledge on *M. citrifolia* by evaluating its potential as an antidiabetic agent through comprehensive biochemical and pharmacological analyses. Findings from this research could support the development of plant-based therapeutic agents, offering a natural and potentially effective alternative for diabetes management.

1.1.1.3 HEAT INDUCED HAEMOLYSIS

Heat-induced hemolysis is a process in which oxidative damage and hemoglobin release occur when red blood cells (RBCs) burst as a result of thermal stress. In diseases like sickle cell disease and other hemolytic illnesses, this feature is especially important. By stabilizing RBC membranes, antioxidants and bioactive substances found in medicinal plants can provide protection against hemolysis.

Because of its high antioxidant content, noni (*Morinda Citrifolia*) has been researched for its potential to stop hemolysis. It has been demonstrated that Noni's flavonoids, phenolic chemicals, and alkaloids shield red blood cells from oxidative damage by scavenging free radicals and lowering lipid peroxidation. According to experimental research, Noni extracts may improve erythrocyte stability, delaying the premature death of cells under heat stress.

According to Reuter *et al*. (2010), oxidative stress has a significant role in the pathophysiology of diseases such as diabetes, cancer, and neurodegenerative disorders as well as cellular damage. Because of their high polyunsaturated fatty acid content and oxygen transport role, red blood cells (RBCs) are especially susceptible to oxidative damage (Percário *et al*., 2012). Heat-induced hemolysis is a paradigm used to evaluate plant extracts' capacity to stabilize membranes and shield cells from inflammation and oxidative damage.

Reactive oxygen species (ROS) are produced in greater quantities under heat stress, which causes lipid peroxidation and erythrocyte membrane rupture (Winterbourn, 2008). By maintaining membrane integrity and scavenging free radicals, medicinal plants with antioxidant qualities, like *Morinda Citrifolia*, may shield erythrocytes from hemolysis (Deng *et al*., 2010). Examining *Morinda Citrifolia* leaf extract's capacity to suppress heat-induced hemolysis will reveal more about its defenses against oxidative damage.

. The integrity of red blood cells (RBCs) is crucial for maintaining oxygen transport and overall physiological balance. However, RBCs are highly susceptible to oxidative stress, which can cause membrane destabilization, leading to haemolysis. Heat-induced haemolysis is a widely used model for assessing the membrane-stabilizing and anti-inflammatory properties of plant extracts, as elevated temperatures can cause RBC lysis by disrupting membrane integrity (Adeyemi & Komolafe, 2020). The ability of bioactive compounds to protect RBCs from heat-induced haemolysis is an important indicator of their potential therapeutic benefits in inflammatory and oxidative stress-related conditions, such as sickle cell disease, anemia, and autoimmune disorders (Olugbade et al., 2021).

Morinda Citrifolia (Noni) has been traditionally used for its medicinal properties, including anti-inflammatory and antioxidant effects, which may contribute to membrane stabilization. Studies have shown that phytochemicals such as flavonoids, alkaloids, and phenolic compounds in *M. citrifolia* exhibit significant free radical-scavenging activities, which can help protect RBCs from oxidative damage (Singh et al., 2019). The presence of bioactive compounds with membrane-stabilizing properties suggests that *M. citrifolia* may help prevent heat-induced haemolysis, thereby reducing inflammation and cellular damage (Rahmat et al., 2021).

Oxidative stress and inflammation play key roles in the pathogenesis of various diseases, including diabetes, cardiovascular disorders, and hemolytic anemia. Since RBC membranes contain polyunsaturated fatty acids, they are particularly vulnerable to peroxidation, which can lead to increased fragility and haemolysis under thermal stress (Kumar & Sharma, 2022). Natural antioxidants from *M. citrifolia* could mitigate this process by enhancing membrane stability, reducing lipid peroxidation, and preventing premature RBC destruction.

Despite traditional claims regarding the medicinal benefits of *M. citrifolia*, there is a need for scientific validation of its role in RBC protection. This study aims to investigate the heat-induced haemolysis protective effects of *M. citrifolia* leaf extract by assessing its ability to stabilize erythrocyte membranes under thermal stress. By understanding its potential as a natural membrane stabilizer, this research could contribute to the development of plant-based therapies for managing hemolytic and inflammatory disorders.

1.1.1.4 ANTI-OXIDANT CAPACITY AND ABTS ASSAY

In order to prevent diseases like diabetes, cardiovascular disease, and neurodegenerative disorders that are linked to oxidative stress, a substance's capacity to neutralize free radicals is essential. For assessing the antioxidant ability of plant extracts, the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) test is a commonly used technique.

One popular technique for assessing the antioxidant potential of plant extracts is the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay (Re *et al.*, 1999). Numerous pathological disorders, such as diabetes, cancer, and cardiovascular diseases, are significantly impacted by oxidative stress (Halliwell & Gutteridge, 2007). By scavenging free radicals, antioxidants protect cells and improve general health.

Oxidative stress is a major contributor to the pathogenesis of various chronic diseases, including diabetes, cardiovascular disorders, neurodegenerative diseases, and cancer. It results from an imbalance between reactive oxygen species (ROS) and the body’s ability to neutralize them through antioxidant defense mechanisms (Halliwell & Gutteridge, 2020). The accumulation of free radicals can lead to cellular damage, lipid peroxidation, protein denaturation, and DNA mutations, making the search for effective antioxidants a critical area of research (Pham-Huy et al., 2022).

Medicinal plants, including *Morinda Citrifolia* (Noni), are rich sources of natural antioxidants such as flavonoids, phenolic compounds, alkaloids, and ascorbic acid, which have been shown to neutralize ROS and reduce oxidative damage (Deng et al., 2019). The antioxidant capacity of *M. citrifolia* has been linked to its therapeutic effects, including its anti-inflammatory, anti-aging, and disease-preventive properties (Singh et al., 2021). However, variations in phytochemical composition due to geographical and environmental factors necessitate standardized evaluation methods to confirm its efficacy.

One of the most reliable methods for assessing antioxidant activity is the ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] assay. The ABTS assay measures the ability of antioxidants to scavenge ABTS radicals, providing a quantitative assessment of a plant extract’s free radical-neutralizing potential (Re et al., 1999). Compared to other assays, ABTS is highly sensitive, applicable to both hydrophilic and lipophilic antioxidants, and allows for the evaluation of complex plant matrices (Prior et al., 2005).

Although *M. citrifolia* has been traditionally recognized for its health benefits, there is a need for scientific validation of its antioxidant capacity using standardized assays like ABTS. This study aims to determine the free radical-scavenging potential of *M. citrifolia* leaf extract through the ABTS assay, providing valuable insights into its efficacy as a natural antioxidant. The findings could contribute to the development of functional foods, nutraceuticals, and pharmaceutical formulations aimed at combating oxidative stress-related diseases.

According to Wang *et al*. (2002), *Morinda Citrifolia 's* polyphenols, flavonoids, and alkaloids have been shown to exhibit potent antioxidant activity. The pharmacological assessment of *Morinda Citrifolia* leaf extract will be aided by the use of the ABTS assay to measure the extract's capacity to scavenge free radicals.

1.1.1.5 TOTAL ALKALOID CONTENT OF *MORINDA CITRIFOLIA*

Alkaloids are a broad class of naturally occurring nitrogen-containing substances that have anti-inflammatory, anti-diabetic, and antibacterial actions, among other bioactive qualities (Cordell, 2017). According to earlier research, *Morinda Citrifolia* contains a number of alkaloids that support its medicinal qualities, including scopoletin, damnacanthal, and xeronine (Kamiya *et al*., 2004).

According to research, Noni has a high concentration of alkaloids, which support its therapeutic benefits. These bioactive substances might have an impact on biological processes related to immunological regulation, redox balance, and glucose metabolism. To determine the plant's potency and its pharmaceutical uses, the total alkaloid content must be measured.

The purpose of this work is to ascertain the total alkaloid content of *Morinda Citrifolia* leaf extract because these bioactive substances may be essential to its antioxidant and anti-diabetic properties. The pharmacological uses of *Morinda Citrifolia* leaves can be better understood by knowing their alkaloid profile.

1.1.1.6 ELEMENTAL ANALYSIS OF *MORINDA CITRIFOLIA*

According to an elemental analysis, *Morinda Citrifolia* has a wide variety of minerals that are essential for preserving metabolic processes and cellular equilibrium. According to certain research, these components help increase the plant's active compounds' bioavailability and effectiveness, which makes it a useful supplement for medicinal uses.

The nutritional and therapeutic qualities of plants are influenced by the materials they receive from the soil, both necessary and non-essential. When evaluating medicinal plants' potential toxicity and therapeutic efficacy, their elemental makeup is crucial (Nwokocha *et al*., 2012). A number of physiological activities, including enzyme activation, neuronal function, and metabolic control, depend on essential elements like calcium (Ca), magnesium (Mg), potassium (K), and zinc (Zn) (Prashanth *et al*., 2015).
The mineral makeup of *Morinda Citrifolia* leaf extract and its possible health advantages—particularly in the treatment of diabetes and antioxidant defense—will be ascertained with the aid of elemental analysis.

1.1.1.7 AMINO ACID PROFILE OF *MORINDA CITRIFOLIA*

Amino acids are essential components of proteins and are involved in many physiological and metabolic functions. The health advantages of medicinal plants may be attributed to the presence of both essential and non-essential amino acids.

At the very end of its branches, the plant bears thick clusters of blooms. Because of their placement across from the leaves, these inflorescences essentially take the place of one leaf in each pair. Only a small number of blooms bloom at any given moment, even though a single head may have 90–100 blossoms. In terms of length and width, the white, tubular, five-lobed blossoms itself are about 15 cm in size.

Numerous biological activities, including as protein synthesis, neurotransmission, and metabolic regulation, depend on amino acids. While non-essential amino acids like glutamate and aspartate aid in the metabolism of cellular energy, essential amino acids like lysine and leucine are necessary for immunological response and growth (Wu, 2013). According to studies, amino acids like glutamine and arginine may improve glucose metabolism and increase insulin production, which may have anti-diabetic effects (Newsholme *et al*., 2014). Understanding the amino acid makeup of *Morinda Citrifolia* leaf extract will help determine its nutritional and medicinal value.

1.1.1.8 DIABETES

A chronic metabolic disease called diabetes mellitus is typified by hyperglycemia brought on by either ineffective insulin action or secretion, or both. With its rising frequency and related consequences, such as cardiovascular illnesses, nephropathy, neuropathy, and retinopathy, it is a significant global public health concern (Khan *et al*., 2020). Type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes mellitus (GDM), and other less prevalent variants are the three main categories of diabetes (American Diabetes Association [ADA], 2021). It is essential to comprehend the pathophysiology, risk factors, and consequences of diabetes in order to create management and treatment plans that work.

Over 537 million persons worldwide had diabetes in 2021, and estimates suggest that number would increase to 783 million by 2045, making diabetes a major global health burden (International Diabetes Federation [IDF], 2021). Type 2 diabetes is becoming far more common as a result of bad eating habits, obesity, and sedentary lifestyles. Low- and middle-income nations, where access to healthcare and diabetes treatment resources is restricted, are disproportionately affected by the condition (Zimmet *et al*., 2016).

Different kinds of diabetes have different pathophysiologies. An autoimmune condition known as type 1 diabetes causes the beta cells in the pancreas to be destroyed, which leaves the patient without enough insulin (Atkinson *et al*., 2014). On the other hand, insulin resistance and increasing beta-cell failure in the pancreas are the main characteristics of type 2 diabetes. Diabetes problems are exacerbated by chronic hyperglycemia, which increases inflammation and oxidative stress (DeFronzo *et al*., 2015).

The development of diabetes is influenced by a number of environmental and genetic variables. Autoimmune reactions and genetic susceptibility (variations in the HLA gene) are risk factors for type 1 diabetes (Knip *et al*., 2020). Obesity, inactivity, poor diet, age, and family history are all linked to type 2 diabetes (Hu, 2011). Pregnancy-related hormonal alterations can result in gestational diabetes, which raises the risk of type 2 diabetes in later life (ADA, 2021).

Microvascular and macrovascular problems result from uncontrolled diabetes. According to Forbes and Cooper (2013), macrovascular problems include peripheral artery disease, stroke, and cardiovascular disorders, whereas microvascular complications include diabetic retinopathy, nephropathy, and neuropathy. Effective glycemic management is necessary because hyperglycemia contributes significantly to these problems through oxidative stress and inflammation.

Glycated hemoglobin (HbA1c) values, random plasma glucose testing, oral glucose tolerance tests (OGTT), and fasting plasma glucose (FPG) levels are used to diagnose diabetes (ADA, 2021). Pharmacotherapy (insulin, metformin, sulfonylureas, etc.), lifestyle changes, and ongoing glucose monitoring are all part of management. Targeting insulin resistance, preserving pancreatic beta cells, and developing novel drug delivery methods are the main goals of emerging treatment approaches. Nathan (2014)

Diabetes mellitus continues to be a major worldwide health concern with wide-ranging socioeconomic effects. To lessen its effects, it is crucial to comprehend its epidemiology, pathophysiology, risk factors, and management techniques. There is hope for better disease control and better patient outcomes from ongoing research on innovative therapeutic strategies and lifestyle changes.

.

1.1.2 STATEMENT OF PROBLEM

Chronic illnesses such as diabetes, oxidative stress-related disorders, and inflammatory conditions remain significant global health challenges, contributing to high morbidity and mortality rates. While synthetic drugs are commonly used for treatment, their prolonged use is often linked to adverse side effects, high costs, and the risk of drug resistance. This has intensified the search for safer, natural alternatives with fewer health risks.

Morinda Citrifolia (Noni) has been traditionally valued for its medicinal properties, including anti-diabetic, antioxidant, and anti-inflammatory effects. However, despite its widespread use in traditional medicine, comprehensive scientific validation of its bioactive compounds and therapeutic efficacy remains insufficient. In particular, there is limited research on its elemental composition, total alkaloid content, antioxidant activity, amino acid profile, membrane-stabilizing effects, and anti-diabetic potential. Investigating these biochemical and pharmacological properties is essential to understanding its possible health benefits and industrial applications.

The mineral composition of *M. citrifolia* leaves is crucial for evaluating both their nutritional significance and potential toxicity, as certain elements support essential biological functions while others may pose health risks at high concentrations. Alkaloids are known for their diverse pharmacological effects, yet their specific contributions to the medicinal properties of *M. citrifolia* require further exploration. Antioxidants play a vital role in neutralizing reactive oxygen species (ROS), and assessing the antioxidant capacity of *M. citrifolia* through the ABTS assay can provide deeper insights into its potential to mitigate oxidative stress-related diseases.

Additionally, amino acids serve as fundamental building blocks of proteins, and analyzing their profile in *M. citrifolia* leaves could reveal its nutritional and medicinal relevance. Heat-induced haemolysis is commonly used to study erythrocyte membrane stability, and examining the protective effects of *M. citrifolia* against thermal stress may highlight its potential role in managing hemolytic conditions. Furthermore, with the increasing prevalence of diabetes, exploring the anti-diabetic properties of *M. citrifolia* is crucial for determining its efficacy in blood sugar regulation and diabetes management.

This study aims to address these gaps by systematically evaluating the elemental composition, alkaloid content, antioxidant activity, amino acid profile, haemolysis protection, and anti-diabetic effects of *M. citrifolia* leaf extract. The findings will provide scientific validation for its traditional uses and contribute to the development of natural, plant-based therapeutic solutions.

1.1.3 JUSTIFICATION OF STUDY

The rising prevalence of chronic diseases, including diabetes, oxidative stress-related disorders, and hemolytic conditions, has intensified the need for effective, affordable, and safer therapeutic alternatives (International Diabetes Federation, 2021; Pham-Huy et al., 2022). While synthetic drugs remain the primary treatment option, their long-term use is often associated with adverse side effects, high costs, and drug resistance, necessitating the exploration of medicinal plants as potential sources of bioactive compounds with therapeutic benefits (Halliwell & Gutteridge, 2020).

Morinda Citrifolia (Noni) has been widely used in traditional medicine for its diverse pharmacological properties, including anti-diabetic, antioxidant, and anti-inflammatory effects (Deng et al., 2019). However, despite its extensive ethnomedicinal applications, scientific validation of its bioactive constituents and therapeutic efficacy remains limited. A comprehensive investigation into its elemental composition, alkaloid content, antioxidant potential, amino acid profile, membrane-stabilizing properties, and anti-diabetic activity is necessary to substantiate its medicinal value and support its potential integration into modern therapeutic applications (Singh et al., 2021).

The elemental composition of *M. citrifolia* leaves is crucial for evaluating their nutritional significance and potential toxicity, as certain minerals are essential for metabolic processes, while excessive levels may pose health risks (Baskar et al., 2020). Alkaloids, known for their diverse biological activities, could significantly contribute to the therapeutic effects of *M. citrifolia*, yet their specific pharmacological roles require further exploration (Rai et al., 2021).

Oxidative stress, a major contributor to chronic diseases, occurs due to an imbalance between free radicals and the body’s antioxidant defense system. Evaluating the antioxidant capacity of *M.*

citrifolia using the ABTS assay provides an effective means of assessing its ability to neutralize free radicals and mitigate oxidative damage (Re et al., 1999; Prior et al., 2005). Additionally, amino acids play a fundamental role in biological functions, and profiling their composition in *M. citrifolia* leaves could offer insights into their nutritional and therapeutic relevance (Friedman, 2018).

Heat-induced haemolysis is a widely accepted model for assessing erythrocyte membrane stability. Investigating the protective effects of *M. citrifolia* against membrane damage could highlight its potential role in managing hemolytic disorders and inflammatory conditions (Tayal et al., 2020). Furthermore, with diabetes affecting millions globally, identifying natural anti-diabetic agents remains a priority. Evaluating the hypoglycemic potential of *M. citrifolia* could provide a plant-based alternative for glycemic control and diabetes management (Hossain et al., 2022).

This study aims to bridge existing knowledge gaps by comprehensively assessing the biochemical and pharmacological properties of *M. citrifolia* leaf extract. Scientific validation of its therapeutic potential could contribute to the development of functional foods, nutraceuticals, and pharmaceutical formulations, ultimately supporting improved healthcare outcomes and alternative treatment strategies.

1.1.4 AIM OF THE STUDY

The study aims to evaluate the biochemical and pharmacological properties of *Morinda Citrifolia* (Noni) leaf extract by analyzing its elemental composition, total alkaloids, antioxidant capacity, amino acid profile, heat-induced haemolysis protection, and anti-diabetic potential. The findings will provide scientific validation of its medicinal properties and potential applications in healthcare and pharmaceutical industries.

1.1.4.1 OBJECTIVES OF THE STUDY

The specific objectives of this study are to:

- determine the elemental composition of *Morinda Citrifolia* leaves – This will help assess their nutritional significance and potential toxicity.
- quantify the total alkaloid content in *Morinda Citrifolia* leaves – Alkaloids are known for their pharmacological properties, and their presence will provide insight into the plant's bioactive potential.
- evaluate the antioxidant capacity of *Morinda Citrifolia* using the ABTS assay – This will determine its ability to neutralize free radicals and mitigate oxidative stress-related diseases.
- analyze the amino acid profile of *Morinda Citrifolia* leaves – Understanding the amino acid composition will highlight the plant's nutritional and therapeutic relevance.
- assess the protective effect of *Morinda Citrifolia* on heat-induced haemolysis – This will determine its role in stabilizing erythrocyte membranes and its potential application in treating hemolytic conditions.
- investigate the anti-diabetic potential of *Morinda Citrifolia* leaf extract – This will provide insight into its hypoglycemic effects and potential use in managing diabetes.
- establish a scientific basis for the traditional medicinal use of *Morinda Citrifolia* – The study aims to support the integration of *M. citrifolia* into modern therapeutic applications based on its bioactive properties..

1.1.5 SCOPE OF THE STUDY

This study focuses on evaluating the biochemical and pharmacological properties of *Morinda Citrifolia* (Noni) leaf extract, emphasizing its elemental composition, alkaloid content, antioxidant capacity, amino acid profile, heat-induced haemolysis protection, and anti-diabetic potential. The research encompasses both qualitative and quantitative analyses to establish the medicinal relevance of *M. citrifolia* by linking its bioactive compounds to its therapeutic effects. Laboratory-based experiments will be conducted to assess its antioxidant activity using the ABTS assay, while its anti-diabetic properties will be evaluated through appropriate biochemical assays. Additionally, the study will investigate the protective effects of the extract on erythrocyte membrane stability under heat-induced stress, providing insights into its potential application in managing oxidative stress and hemolytic conditions. The findings from this study will contribute to the scientific validation of *M. citrifolia* as a medicinal plant, offering a basis for its possible integration into natural therapeutic interventions and pharmaceutical formulations.

1.1.6 SIGNIFICANCE OF THE STUDY

This study is significant as it provides scientific validation for the medicinal potential of *Morinda Citrifolia* (Noni) leaves, which have been widely used in traditional medicine but lack sufficient empirical support. By evaluating its elemental composition, alkaloid content, antioxidant activity, amino acid profile, membrane-stabilizing effects, and anti-diabetic potential, the research offers valuable insights into the plant's pharmacological and nutritional relevance.

The study's findings could contribute to the development of plant-based therapies for managing oxidative stress, diabetes, and hemolytic conditions, potentially offering safer and more affordable alternatives to synthetic drugs. Additionally, understanding the antioxidant properties of *M. citrifolia* could help in its application in the nutraceutical and functional food industries, where natural compounds are increasingly sought after for disease prevention and health promotion.

From a scientific perspective, this research expands the knowledge of *M. citrifolia*'s bioactive compounds and their interactions, paving the way for further studies on its mechanisms of action. Moreover, it supports the conservation and utilization of medicinal plants by providing a basis for their standardization in modern medicine. The study also has implications for pharmacognosy, natural product chemistry, and drug discovery, reinforcing the importance of traditional medicinal plants in contemporary healthcare.

1.2 LITERATURE REVIEW

Fig 1.2 Morinda Citrifolia

1.2.1 MEDICINAL HERBS AND THEIR BIOACTIVE COMPOUNDS

For ages, traditional medicine has employed medicinal plants to treat and prevent a wide range of illnesses. Nearly 80% of people worldwide, particularly in developing nations, rely on plant-based treatments for primary healthcare, according to the World Health Organization. (WHO, 2019). By separating and identifying the bioactive chemicals that give many plants their therapeutic effects, modern research has confirmed that many plants have medicinal qualities (Rates, 2001).

Numerous chemicals produced from plants have been used as the basis for pharmaceutical medications like aspirin, quinine, and metformin thanks to advancements in phytochemistry and pharmacology (Atanasov *et al*., 2015).

The secondary metabolites that medicinal plants produce are what give them their pharmacological effects. These substances fall into a number of main categories, which are;

Alkaloids; Alkaloids are nitrogen-containing compounds with significant pharmacological effects (Cushnie *et al*., 2014). They exhibit anti-diabetic, anti-inflammatory, analgesic, and antimicrobial properties.Examples are Metformin (derived from Galega officinalis), a widely used anti-diabetic drug (Bailey & Day, 2004, and Quinine (from Cinchona species), used to treat malaria (Rogerson, 2010).

Flavonoids; Flavonoids are polyphenolic compounds with strong antioxidant, anti-inflammatory, and cardioprotective effects (Panche *et al*., 2016). They help neutralize free radicals and reduce oxidative stress, which is linked to diabetes, cancer, and neurodegenerative diseases (Kumar & Pandey, 2013).

Terpenoids; Terpenoids have antimicrobial, anti-cancer, and anti-inflammatory properties (Thoppil & Bishayee, 2011). Some exhibit hypoglycemic effects by influencing insulin secretion and glucose metabolism. Examples are Curcumin (from Curcuma longa), known for its anti-inflammatory and antioxidant properties (Gupta *et al*., 2013).

Saponins; Saponins are glycosides with immune-boosting, anti-diabetic, and cholesterol-lowering properties (Francis *et al*., 2002). Found in Moringa oleifera and Panax ginseng, they are used in traditional medicine for metabolic disorders.

Phenolic Compounds; Potent antioxidants that prevent oxidative damage to cells (Rice-Evans *et al*., 1997). Play a key role in anti-diabetic, anti-aging, and neuroprotective functions.

Glycosides; They are Sugar-containing bioactive compounds with cardioprotective and anti-diabetic effects (Van Wyk & Wink, 2018). Examples are Stevioside (from Stevia rebaudiana), a natural sweetener with glucose-lowering effects (Chatsudthipong & Muanprasat, 2009).

1.2.1.1 PHARMACOLOGICAL IMPORTANCE OF MEDICINAL PLANTS

a. Anti-Diabetic Properties

- Medicinal plants such as Momordica charantia, Moringa oleifera, and Gymnema sylvestre contain bioactive compounds that help:

  i)Lower blood glucose levels.

  ii)Improve insulin sensitivity.

  iii)Reduce oxidative stress associated with diabetes (Patel *et al*., 2012).

b. Antioxidant Properties

- Oxidative stress contributes to aging, cancer, diabetes, and neurodegenerative diseases (Halliwell, 2007).
- Polyphenols, flavonoids, and carotenoids from medicinal plants neutralize free radicals and reduce cellular damage (Kumar *et al*., 2013).

c. Antimicrobial and Antiviral Properties

- Many medicinal plants have broad-spectrum antimicrobial activity due to their alkaloids, tannins, and flavonoids (Cowan, 1999).

- Example: Garlic (Allium sativum) contains allicin, which fights bacterial and fungal infections (Ankri & Mirelman, 1999).

d. Anti-Inflammatory Effects

- Chronic inflammation is linked to diseases like arthritis, cancer, and diabetes (Aggarwal *et al*., 2006).
- Medicinal plants contain anti-inflammatory compounds that inhibit inflammatory pathways.

e. Anti-Cancer Potential

- Some medicinal plants show potential for cancer prevention and treatment (Newman & Cragg, 2016).
- Example: Vinblastine and vincristine (from Catharanthus roseus), used in chemotherapy (Nobili *et al*., 2009).

f. Neuroprotective Effects

- Medicinal plants help protect the brain from neurodegenerative diseases like Alzheimer's and Parkinson's (Howes & Perry, 2011).
- Example: Bacopa monnieri (Brahmi) enhances learning and neuroprotection.

1.2.1.2 ROLE OF MEDICINAL PLANTS IN DRUG DEVELOPMENT

Many modern pharmaceuticals are derived from medicinal plants (Atanasov *et al*., 2015):

- **Aspirin** (from Salix alba – willow bark).
- **Metformin** (from Galega officinalis – goat's rue) for diabetes.
- **Vincristine and Vinblastine** (from Catharanthus roseus) for cancer treatment.
- **Morphine** (from Papaver somniferum – opium poppy) for pain relief.

The antioxidant, anti-inflammatory, antibacterial, anti-diabetic, and anti-cancer properties of *Morinda Citrifolia* are attributed to its diverse range of bioactive components, which include terpenoids, alkaloids, flavonoids, and phenolic compounds (Wang *et al*., 2002). There have been reports of notable medical benefits from the plant's leaves, fruits, roots, and seeds. (Dussossoy & colleagues, 2011)

1.2.2 ANTIDIABETIC ACTIVITY OF *MORINDA CITRIFOLIA*

Hyperglycemia, or elevated blood glucose levels, is a hallmark of diabetes mellitus, a chronic metabolic disease brought on by either insulin resistance, inadequate insulin synthesis, or both (American Diabetes Association, 2022). Globally, the prevalence of diabetes has been increasing, and its consequences include cardiovascular illnesses, retinopathy, nephropathy, and neuropathy (Khan *et al*., 2020).

Because of their wide range of bioactive chemicals and fewer side effects, herbal medications have drawn interest as complementary or alternative therapy for the management of diabetes (Patel *et al*., 2012). Through a variety of mechanisms, such as improving insulin sensitivity, lowering oxidative stress, modifying carbohydrate metabolism, and shielding pancreatic β-cells, *Morinda Citrifolia* (Noni), a medicinal plant used in traditional medicine, has shown notable antidiabetic properties (Deng *et al*., 2011).

1.2.2.1 MECHANISMS OF ANTIDIABETIC ACTION

a. Reduction of Blood Glucose Levels

- Studies have demonstrated that *Morinda Citrifolia* significantly reduces fasting blood glucose and HbA1c levels in diabetic animal models (West *et al*., 2009).

- A study by Wang *et al*. (2002) showed that administration of noni fruit extract to diabetic rats led to a 30% reduction in blood glucose levels over a four-week period.

b. Enhancement of Insulin Sensitivity

- *Morinda Citrifolia* has been found to enhance insulin receptor signaling, improving glucose uptake and utilization in insulin-resistant cells (Yang *et al*., 2010).
- A study on human diabetic patients showed that regular consumption of noni juice improved insulin sensitivity and reduced insulin resistance markers (Deng *et al*., 2011).

c. Protection of Pancreatic β-Cells

- Oxidative stress is a major contributor to pancreatic β-cell damage in diabetes. The high antioxidant content in *Morinda Citrifolia* helps neutralize free radicals, thereby protecting these cells (Kamiya *et al*., 2008).
- Damnacanthal and scopoletin have shown anti-apoptotic effects, preventing β-cell death (Hiramatsu *et al*., 1993).

d. Inhibition of Carbohydrate-Digesting Enzymes

- *Morinda Citrifolia* extracts inhibit α-amylase and α-glucosidase, enzymes responsible for breaking down carbohydrates into glucose. This results in delayed glucose absorption and lower postprandial glucose levels (Liu *et al*., 2014).
- This mechanism is similar to that of acarbose, a pharmaceutical drug used to manage diabetes (Dussossoy *et al*., 2011).

e. Modulation of Lipid Profile

- Diabetes is often associated with dyslipidemia, characterized by high LDL cholesterol and triglycerides.
- *Morinda Citrifolia* has been found to reduce LDL cholesterol and increase HDL cholesterol, improving overall metabolic health (Patel *et al*., 2012).

1.2.2.2 PRE-CLINICAL AND CLINICAL EVIDENCE

Animal Studies

- • In a study on diabetic rats, Deng *et al*. (2011) found that administering noni leaf extract significantly decreased fasting blood glucose, enhanced insulin sensitivity, and raised levels of pancreatic antioxidant enzymes.

- Rats given noni juice had better lipid metabolism and lower glucose levels than the control group, according to research by West *et al*. (2009).

Human Clinical Trials

• Patients with type 2 diabetes who consumed noni juice daily for eight weeks saw a significant reduction in their fasting blood sugar levels, according to a clinical trial by Wang *et al*. (2002).

• In a placebo-controlled study, Mahattanadul *et al*. (2011) found that supplementing diabetes patients with noni fruit extract increased their glucose tolerance and decreased their HbA1c levels.

1.2.2.3 POTENTIAL APPLICATIONS IN DIABETES MANAGEMENT

Given its multiple mechanisms of action, *Morinda Citrifolia* can be incorporated into diabetes management in the following ways:

1. As a Functional Food
   - Noni juice and leaf extracts can be consumed as part of a diabetic diet to aid in blood sugar regulation.
2. As a Complementary Therapy
   - *Morinda Citrifolia* can be used alongside conventional diabetes medications to enhance their effects and reduce side effects.
3. As a Pharmaceutical Drug Candidate
   - Bioactive compounds such as damnacanthal and scopoletin from M. citrifolia could be further developed into plant-based diabetes drugs.

1.2.3 ANTIOXIDANT ACTIVITY: ABTS ASSAY

According to Halliwell (2012), oxidative stress is a major factor in the development of a number of chronic illnesses, including as diabetes, heart disease, cancer, and neurological conditions. Antioxidants are substances that prevent oxidative damage to cells by neutralizing free radicals. Due in significant part to its phenolic components, flavonoids, and alkaloids, *Morinda Citrifolia* (noni) is well known for having strong antioxidant properties (Deng *et al*., 2011).

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay is one of the most popular techniques for assessing the ability of plant extracts to scavenge free radicals among a variety of antioxidant assays (Re *et al*., 1999). The ability of M. citrifolia extracts to neutralize ABTS radicals, a sign of their antioxidant potential, is measured using the ABTS radical cation ($ABTS^{\bullet+}$) scavenging assay.

Deng *et al*. (2011) used an ABTS assay on extracts of *Morinda Citrifolia* and found that the TEAC values were substantial antioxidants, ranging from 1.5 to 2.5 mM Trolox equivalents. Phenolic-rich fractions demonstrated 80–90% $ABTS^{\bullet+}$ radical inhibition, which is equivalent to synthetic antioxidants like butylated hydroxytoluene (BHT), according to a 2011 study by Dussossoy *et al*. that assessed the ABTS scavenging capacity of noni fruit juice.

West *et al*. (2009) discovered that while *Morinda Citrifolia* leaves contain more polyphenols than its fruit, they have greater ABTS radical scavenging action.

Morinda Citrifolia's antioxidant potency in the ABTS assay was on par with that of vitamin E and green tea (Deng *et al*., 2011).
When noni juice was compared to commercial antioxidants, Mahattanadul *et al*. (2011) discovered that it was just as effective as ascorbic acid at scavenging $ABTS^{\bullet+}$ radicals.

1.2.3.1 POTENTIAL HEALTH BENEFITS OF ABTS ANTIOXIDANT ACTIVITY IN *MORINDA CITRIFOLIA*

1. Defense Against Diseases Associated with Oxidative Stress o *Morinda Citrifolia*'s ability to scavenge ABTS radicals points to its potential to prevent free radical-induced cellular damage, lowering the risk of diabetes, cardiovascular disease, and neurological disorders (Halliwell, 2012).

2. Anti-Inflammatory Effects o By counteracting oxidative stress, a key contributor to chronic illnesses including asthma and arthritis, the polyphenols and flavonoids in *Morinda Citrifolia* help to reduce inflammation (Dussossoy *et al*., 2011).

3. Benefits for Skin and Anti-Aging *Morinda Citrifolia* is utilized in cosmetic formulations to protect skin from oxidative damage, lowering wrinkles, pigmentation, and skin aging because of its strong ABTS antioxidant activity (Yang *et al*., 2010).

4. Neuroprotection o Research indicates that noni's ABTS radical scavenging ability lowers the risk of Alzheimer's and Parkinson's illnesses by shielding neurons from oxidative damage (Mahattanadul *et al*., 2011).

Because of its high amount of polyphenols, flavonoids, anthraquinones, and vitamin C, *Morinda Citrifolia* has shown remarkable ABTS radical scavenging activity. The ABTS assay is a very dependable way to assess the antioxidant capacity of medicinal plants. According to studies, noni extracts have a great capacity to neutralize free radicals, which makes them an effective natural antioxidant for avoiding diseases linked to oxidative stress. Its traditional usage in cardiovascular health, neuroprotection, and anti-aging is supported by its high antioxidant capacity.

1.2.4 TOTAL PHENOLIC CONTENT OF *MORINDA CITRIFOLIA*

Plant secondary metabolites called phenolic compounds are essential for their antibacterial, anti-inflammatory, and antioxidant properties (Balasundram *et al*., 2006). As natural scavengers of free radicals, these substances shield cells from oxidative damage and support the therapeutic benefits of medicinal plants (Halliwell, 2012).

Noni, also known as *Morinda Citrifolia*, is well known for having a high phenolic content, which supports its potent antioxidant properties. Environmental circumstances, extraction technique, and plant parts (leaves, fruit, and roots) all affect the total phenolic content (TPC) of noni (Dussosoy *et al*., 2011). According to studies, noni leaves are a powerful source of natural antioxidants since they have a higher phenolic content than the fruit (Mohd Zin *et al*., 2002).

1.2.4.1 DETERMINATION OF TOTAL PHENOLIC CONTENT IN *MORINDA CITRIFOLIA*

a. Folin-Ciocalteu Assay

The most used technique for determining the total phenolic content (TPC) of plant extracts is the Folin-Ciocalteu (FC) test. It is predicated on the fact that phenolic chemicals reduce the Folin-Ciocalteu reagent, resulting in a colorimetric reaction that is detected at 765 nm (Singleton *et al*., 1999).

b. Factors Affecting TPC in *Morinda Citrifolia*

Several factors influence the **total phenolic content** in noni:

• Plant Part: According to West *et al*. (2009), leaves have a greater TPC than fruit and roots.
• Extraction Solvent: According to Mohd Zin *et al*. (2002), ethanol and methanol extracts had greater TPC than water extracts.

• Maturity Stage: According to Deng *et al*. (2011), unripe noni fruit has a greater TPC than fully ripened fruit.

1.2.4.2 Major Phenolic Compounds Identified in *Morinda Citrifolia*

Studies have identified several bioactive phenolic compounds in *Morinda Citrifolia,* including:

a. Gallic Acid

- A potent antioxidant found in noni leaves and fruit.
- Known for its anti-inflammatory, antimicrobial, and anti-cancer properties (Dussossoy *et al*., 2011).

b. Chlorogenic Acid

- Found abundantly in noni fruit and leaves**.**
- Known for its anti-diabetic, anti-obesity, and neuroprotective effects **(**Yang *et al*., 2010**).**

c. Caffeic Acid

- Exhibits strong antioxidant activity, protecting against lipid peroxidation.
- Enhances anti-inflammatory and immune-boosting properties **(**Mohd Zin *et al*., 2002**).**

d. Rutin

- A flavonoid that protects against oxidative stress and cardiovascular diseases.
- Found in noni leaves, contributing to its high total phenolic content **(**West *et al*., 2009**).**

1.2.4.3 COMPARATIVE ANALYSIS OF TOTAL PHENOLIC CONTENT IN *MORINDA CITRIFOLIA*

a. Phenolic Content in Different Plant Parts

Plant Part	Total Phenolic Content (mg GAE/g dry weight)
Leaves	45–70 mg GAE/g
Unripe Fruit	30–50 mg GAE/g
Ripe Fruit	10–25 mg GAE/g
Roots	20–40 mg GAE/g

- Leaves have the highest TPC, followed by unripe fruit, roots, and ripe fruit.
- The higher phenolic content in leaves makes them a better source of antioxidants than the fruit.

b. Comparative Studies with Other Medicinal Plants

- Morinda. citrifolia has higher TPC than Aloe vera but lower than green tea and Camellia sinensis (West *et al*., 2009).
- The antioxidant activity of noni leaf extract is comparable to that of grape seed extract and vitamin C (Deng *et al*., 2011).

1.2.4.4 Health Benefits of Total Phenolic Content in *Morinda Citrifolia*

a. Antioxidant Activity

- Phenolics in Morinda. citrifolia help neutralize free radicals**,** reducing the risk of oxidative stress-related diseases **(**Halliwell, 2012**).**
- **The** ABTS and DPPH assays confirm that noni extracts exhibit strong antioxidant properties **(**Dussossoy *et al*., 2011**).**

b. Anti-Diabetic Potential

- Chlorogenic and caffeic acids in noni enhance insulin sensitivity and reduce blood sugar levels **(**Yang *et al*., 2010**).**
- Phenolic compounds inhibit α-glucosidase**,** reducing postprandial glucose levels **(**Deng *et al*., 2011**).**

c. Anti-Inflammatory and Immune Modulation

- Rutin and gallic acid reduce pro-inflammatory cytokines**,** helping in autoimmune disorders and arthritis **(**Mohd Zin *et al*., 2002**).**
- Phenolics modulate immune response**,** making noni effective against chronic infections **(**West *et al*., 2009**).**

d. Antimicrobial Properties

• According to Dussossoy *et al*. (2011), *Morinda Citrifolia* phenolic extracts have antibacterial properties against Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus.

• Noni is efficient against strains of bacteria that are resistant to antibiotics because gallic acid and flavonoids break down bacterial biofilms (Yang *et al*., 2010).

Morinda Citrifolia's anti-inflammatory, antibacterial, and antioxidant qualities are mostly attributed to its total phenolic content. Noni leaves are a superior source of bioactive components than the fruit since they have the highest concentration of phenolic compounds. Research demonstrates that noni extracts' high TPC enhances their therapeutic potential in the treatment of microbial infections, diabetes, and oxidative stress.

1.2.5 TOTAL ALKALOIDS OF *MORINDA CITRIFOLIA*

Alkaloids are a broad class of naturally occurring nitrogen-containing substances that have anti-inflammatory, anti-microbial, antioxidant, and anti-diabetic actions, among other bioactive qualities (Evans, 2009). These secondary metabolites are important for both medicinal and defense purposes for plants. The high alkaloid content of *Morinda Citrifolia*, often known as noni, is well-established and adds to its therapeutic qualities (Deng *et al*., 2011).

In Polynesian, Indian, and Southeast Asian medicine, noni has long been used to treat a variety of conditions, including inflammation, diabetes, infections, and high blood pressure (West *et al*., 2009). The plant component, extraction technique, and ambient conditions all affect M. citrifolia's overall alkaloid content. Research demonstrates that noni leaves and roots have greater levels of alkaloids than the fruit (Sarfaraz *et al*., 2020).

1.2. 5.1 DETERMINATION OF TOTAL ALKALOIDS IN *MORINDA CITRIFOLIA*

a. Extraction and Quantification Methods

The total alkaloid content in *Morinda Citrifolia* is commonly analyzed using:

1. Acid-Base Extraction Method: Alkaloids are precipitated by adjusting the pH after being extracted using acidic solutions (Harborne, 1998).

2. Spectrophotometric Analysis:The absorbance at 470 nm is measured using the Bromocresol Green (BCG) method, which is utilized for quantitative alkaloid analysis (Fadhil *et al*., 2020).

3. Individual alkaloids can be precisely separated and quantified using High-Performance Liquid Chromatography (HPLC) (Younos *et al*., 1990).

b. Factors Influencing Total Alkaloid Content

1. According to West *et al*. (2009), the largest concentration of alkaloids is found in the roots and leaves of the plant, followed by the unripe fruit.

2. Maturity Stage: According to Deng *et al*. (2011), young noni plants have higher levels of alkaloids than mature plants.

3.Extraction Solvent: According to Sarfaraz *et al*. (2020), ethanol and methanol extracts provide greater alkaloid concentrations than water extracts.

1.2.5.2 Major Alkaloids Identified in *Morinda Citrifolia*

Several bioactive alkaloids have been identified in noni, contributing to its pharmacological effects.

a. Xeronine

- The most widely studied alkaloid in noni.
- Proposed by Ralph Heinicke (1985) as a pro-xeronine precursor**,** which helps activate enzymes and proteins in the body.
- Plays a role in immune modulation, pain relief, and cell repair (Heinicke, 1985).

b. Damnacanthal

- **A** potent anti-cancer alkaloid found in noni root and leaves.
- Shown to inhibit Ras oncogene activation**,** making it a promising candidate for cancer treatment **(**Hirazumi & Furusawa, 1999**).**
- Exhibits antiviral, antibacterial, and anti-inflammatory effects **(**Younos *et al*., 1990**).**

c. Scopoletin

- Although primarily a coumarin**,** scopoletin shares alkaloid-like properties.

- Known for its antihypertensive, anti-inflammatory, and neuroprotective effects **(**Deng *et al*., 2011**).**

d. Anthraquinone Alkaloids (Morindone and Alizarin)

- Found in roots and bark of noni.
- Exhibit strong antibacterial and antifungal activities (West *et al*., 2009).

1.2.5.3 Health Benefits of Total Alkaloids in *Morinda Citrifolia*

a. Anti-Diabetic Effects

- Alkaloids in noni enhance insulin secretion and glucose metabolism (Sarfaraz *et al*., 2020).
- Xeronine and scopoletin help regulate blood sugar levels and insulin resistance (Deng *et al*., 2011).

b. Antimicrobial Properties

- Damnacanthal and anthraquinones in noni inhibit bacterial growth**,** including Staphylococcus aureus, E. coli, and Pseudomonas aeruginosa **(**Younos *et al*., 1990**).**
- Exhibits antifungal activity against Candida species **(**West *et al*., 2009**).**

c. Anti-Cancer Potential

- Damnacanthal inhibits tumor growth by blocking oncogene activation **(**Hirazumi & Furusawa, 1999**).**
- Alkaloid-rich noni extracts have shown cytotoxic effects on lung, liver, and breast cancer cells **(**Deng *et al*., 2011**).**

d. Neuroprotective and Anti-Inflammatory Effects

- Xeronine and scopoletin help regulate neurotransmitter activity**,** reducing symptoms of Alzheimer's and Parkinson's disease **(**West *et al*., 2009**).**

- Damnacanthal suppresses inflammatory cytokines**,** making it beneficial for arthritis and autoimmune disorders **(**Sarfaraz *et al*., 2020**).**

The anti-diabetic, antibacterial, anti-inflammatory, and neuroprotective benefits of *Morinda Citrifolia* are mostly attributed to its total alkaloid concentration. The strongest components for therapeutic purposes are the roots and leaves because they have the largest concentrations of alkaloids.

1.2.6 ELEMENTAL ANALYSIS OF *MORINDA CITRIFOLIA*

Since it establishes the existence and concentration of important and trace elements in plants, elemental analysis is a crucial component of phytochemical study. Although the bioactive components of *Morinda Citrifolia* (noni) have been the subject of much research, its elemental makeup is equally important because of its use in human nutrition, medicine, and toxicity assessment (Deng *et al*., 2011). While trace elements like copper, manganese, and selenium support enzymatic reactions and antioxidant defense systems, essential minerals like calcium, magnesium, potassium, zinc, and iron are essential for biological functions (West *et al*., 2009).

The plant part (leaves, fruit, roots, or bark), soil type, location, and environmental conditions all affect *Morinda Citrifolia*'s elements makeup (Mahattanatawee *et al*., 2006). To ascertain the elemental profile of noni, sophisticated methods including X-ray fluorescence (XRF), inductively coupled plasma mass spectrometry (ICP-MS), and atomic absorption spectroscopy (AAS) are frequently employed (Palu *et al*., 2008).

1.2.6.1 METHODS OF ELEMENTAL ANALYSIS IN *MORINDA CITRIFOLIA*

a. Sample Preparation

• Grinding and Drying: o To eliminate moisture, fresh *Morinda Citrifolia* materials (leaves, fruit, roots, or bark) are dried at 40–60°C before being finely milled into a powder (Deng *et al*., 2011).

• Acid Digestion: To break down organic matter and release the components into a solution, the plant powder is digested using hydrochloric acid (HCl), nitric acid (HNO_3), or a combination of the two (Palu *et al*., 2008).

b. Methods of Analysis

- Atomic Absorption Spectroscopy (AAS):
    - Used for detecting heavy metals like lead (Pb), cadmium (Cd), and mercury (Hg) at trace levels (West *et al*., 2009).
- Inductively Coupled Plasma Mass Spectrometry (ICP-MS):
    - Highly sensitive and used for measuring trace elements such as selenium (Se) and manganese (Mn) (Mahattanatawee *et al*., 2006).
- X-ray Fluorescence (XRF) Spectroscopy:
    - Non-destructive technique for analyzing major minerals like calcium (Ca), potassium (K), and magnesium (Mg) (Sarfaraz *et al*., 2020).

1.2.6.2 HEALTH BENEFITS OF THE ELEMENTAL ANALYSIS OF *MORINDA CITRIFOLIA*

a. Bone Health and Muscle Function

- High levels of calcium (Ca) and magnesium (Mg) support bone density, muscle relaxation, and nerve signaling **(**Deng *et al*., 2011**).**
- Potassium (K) prevents muscle cramps and supports cardiovascular function **(**Mahattanatawee *et al*., 2006**).**

b. Antioxidant and Immune Function

- Zinc (Zn) and Selenium (Se) boost immune responses and help in wound healing **(**West *et al*., 2009**).**
- Manganese (Mn) is essential for superoxide dismutase (SOD) enzyme activity**,** which neutralizes free radicals **(**Palu *et al*., 2008**).**

c. Blood Pressure Regulation

- Potassium (K) reduces hypertension by counteracting excess sodium (Na) in the body **(**Sarfaraz *et al*., 2020**).**

d. Prevention of Anemia and Fatigue

- Iron (Fe) is crucial for hemoglobin production**,** preventing iron-deficiency anemia **(**West *et al*., 2009**).**

1.2.6.3 Toxicity and Safety Evaluation

a. Potential Risks of Heavy Metals

- Studies confirm that noni plants grown in polluted environments may accumulate lead (Pb) and cadmium (Cd) **(**WHO, 2007**).**
- Regular consumption should be monitored to avoid heavy metal toxicity **(**Deng *et al*., 2011**).**

b. Recommended Daily Intake (RDI)

- The mineral content of noni is within safe dietary limits for most consumers **(**Palu *et al*., 2008**).**
- However, excessive intake may lead to mineral imbalances, especially sodium (Na) and potassium (K) **(**Sarfaraz *et al*., 2020**).**
- *Morinda Citrifolia*'s rich composition of vital macro and microelements, which support its nutritional and therapeutic advantages, is revealed by elemental analysis. It supports immune system function, antioxidant defense systems, and bone health as a valuable source of calcium, magnesium, potassium, iron, zinc, and selenium. Although noni cultivated in polluted areas are generally safe to eat, it is important to keep an eye out for heavy metal poisoning.

1.2.7 GAS CHROMATOGRAPHY MASS SPECTROSCOPY (GC-MS) ANALYSIS OF *MORINDA CITRIFOLIA*

A strong analytical method that is frequently used for the detection and measurement of volatile and semi-volatile substances in plant extracts is gas chromatography-mass spectroscopy (GC-MS). Alkaloids, flavonoids, terpenes, fatty acids, and phenolic compounds are among the complex variety of bioactive phytochemicals found in *Morinda Citrifolia* (noni); many of these may be efficiently examined using GC-MS (Deng *et al*., 2011; Potterat & Hamburger, 2007).

GC-MS provides high sensitivity, selectivity, and accuracy in profiling the phytochemical constituents of *Morinda Citrifolia*, making it a crucial tool in pharmacological and nutraceutical research **(**West *et al*., 2009**).**

GC-MS combines two powerful analytical techniques:

- Gas Chromatography (GC): Separates the volatile components of a mixture based on their boiling points and polarity.
- Mass Spectrometry (MS): Identifies and quantifies the separated compounds based on their molecular weight and fragmentation pattern (Palu *et al*., 2008).

The technique is particularly useful for analyzing essential oils, volatile organic compounds (VOCs), and secondary metabolites in plant extracts (Mahattanatawee *et al*., 2006).

A comprehensive phytochemical profile of *Morinda Citrifolia* is provided by GC-MS analysis, which identifies terpenoids, fatty acids, alkaloids, and flavonoids with antibacterial, anti-inflammatory, antidiabetic, and antioxidant qualities. This data demonstrates noni's potential for pharmacological and nutraceutical uses while also confirming its traditional therapeutic use.

1.2.8 PHYTOCHEMICAL SCREENING AND EXTRACTION

In order to discover and separate bioactive chemicals from medicinal plants, phytochemical screening and extraction are essential procedures. Alkaloids, flavonoids, anthraquinones, phenolics, terpenoids, and glycosides are all present in *Morinda Citrifolia* (Noni), a well-known traditional medicinal plant, and they all support its therapeutic qualities (Deng *et al*., 2011; Potterat & Hamburger, 2007).

While extraction techniques maximize the recovery of these chemicals for biological and pharmacological investigations, phytochemical screening offers both qualitative and quantitative information on the plant's secondary metabolites (West *et al*., 2009).

1.2.8.1 EXTRACTION TECHNIQUES FOR PHYTOCHEMICALS IN *MORINDA CITRIFOLIA*

The choice of extraction method significantly affects the yield and potency of bioactive compounds in *Morinda Citrifolia* **(**Deng *et al*., 2011; Potterat & Hamburger, 2007**).**

a. Solvent Extraction Methods

i. Maceration

- Procedure**:** Plant material is soaked in a solvent (ethanol, methanol, or water) for 24-72 hours at room temperature.
- Advantages: Simple, cost-effective**.**
- Disadvantages: Long extraction time, lower efficiency.

Sasidharan *et al*. (2011)

ii. Soxhlet Extraction

- Procedure**:** Plant material is placed in a thimble and extracted using continuous solvent recycling.
- Advantages**:** High efficiency, good yield of bioactives.
- Disadvantages**:** Requires high temperatures, which may degrade some compounds.

Harborne **(**1998**)**

iii. Ultrasonic-Assisted Extraction (UAE)

- Procedure**:** Uses ultrasound waves to break plant cell walls, enhancing solvent penetration.
- Advantages**:** Fast, high yield, retains heat**-**sensitive compounds**.**
- Disadvantages**:** Requires specialized equipment**.**

iv. Supercritical Fluid Extraction (SFE)

- Procedure**:** Uses CO_2 at supercritical conditions to extract non-polar compounds.
- Advantages**:** Selective, solvent-free, environmentally friendly**.**
- Disadvantages**:** Expensive equipment.

b. Factors Influencing Extraction Efficiency

Several factors affect the yield and purity of bioactive compounds from *Morinda Citrifolia* **(**West *et al*., 2009**):**

- Solvent Polarity: Affects the solubility of different classes of phytochemicals**.**
- Temperature and Time**:** Excessive heat can degrade phenolics and flavonoids.
- Particle Size: Smaller particles increase surface area, improving extraction.
- Solvent-to-Sample Ratio**:** Higher ratios improve extraction efficiency.

1.2.8.2 APPLICATIONS OF EXTRACTED PHYTOCHEMICALS FROM *MORINDA CITRIFOLIA*

The extracted phytochemicals have diverse applications in medicine, food, and cosmetics:

Bioactive Compound	**Therapeutic Use**
Xeronine	Cellular repair, enzyme activation
Damnacanthal	Anti-cancer, anti-microbial
Scopoletin	Antioxidant, anti-inflammatory
Anthraquinones	Laxative, antifungal

Morinda Citrifolia's extensive bioactive profile, which includes terpenoids, alkaloids, flavonoids, and anthraquinones, has been validated by phytochemical screening and extraction. The yield of heat-sensitive phytochemicals is increased by sophisticated extraction methods including supercritical fluid extraction and ultrasound-assisted extraction. These bioactive substances support *Morinda Citrifolia*'s status as a valued medical plant by having uses in pharmaceutical, nutraceutical, and cosmetic products.

1.2.9 AMINO ACID PROFILE OF *MORINDA CITRIFOLIA*

As the building blocks of proteins, amino acids are vital macromolecules that are involved in enzyme function, cell metabolism, and physiological processes (Wu, 2013). The nutritional and pharmacological advantages of *Morinda Citrifolia* (Noni), a well-known medicinal plant, are attributed to its varied profile of essential and non-essential amino acids (Deng *et al*., 2011; Potterat & Hamburger, 2007).

Morinda Citrifolia's nutritional value, potential for treatment, and function in metabolic pathways can all be understood using amino acid profiling (West *et al*., 2009). These amino acids strengthen the plant's immune-stimulating, antioxidant, and anti-inflammatory qualities (Palu *et al*., 2008).

The amino acids in *Morinda Citrifolia* play a significant role in its medicinal and nutritional benefits**:**

a. Antioxidant and Anti-inflammatory Properties

- Tryptophan and Methionine contribute to glutathione synthesis, a powerful antioxidant that reduces oxidative stress and inflammation **(**Palu *et al*., 2008).
- Glutamine and Glycine help modulate immune response, reducing inflammation in chronic diseases **(**Wu, 2013**).**

b. Wound Healing and Collagen Synthesis

- Lysine and Proline enhance collagen formation**,** aiding tissue repair and wound healing **(**Potterat & Hamburger, 2007**).**
- Serine and Alanine support cell regeneration and skin health **(**West *et al*., 2009**).**

c. Neuroprotective and Cognitive Benefits

- Tyrosine and Phenylalanine act as precursors for dopamine and norepinephrine, improving cognitive function and mood regulation (Deng *et al*., 2011).
- Histidine contributes to neurotransmitter function and brain health (Wu, 2013).

d. Metabolic and Cardiovascular Health

- Arginine helps regulate blood pressure through nitric oxide production (Mahattanatawee *et al*., 2006).
- Leucine, Isoleucine, and Valine (Branched-Chain Amino Acids - BCAAs) improve muscle metabolism and energy production (West *et al*., 2009).

Using sophisticated analytical methods, amino acid profiling entails the identification and measurement of individual amino acids in plant extracts (Sasidharan *et al*., 2011).

a. High-Performance Liquid Chromatography (HPLC)

Procedure:

Sample hydrolysis with 6N HCl at 110°C for 24 hours. Derivatization using o-phthaldialdehyde (OPA) reagent. Separation via reverse-phase HPLC with UV detection (Harborne, 1998).

b. Gas Chromatography-Mass Spectrometry (GC-MS)

Procedure:

Amino acids are converted into volatile derivatives using N-acylation or silylation, and then analyzed using mass spectrometry (MS) for structural confirmation (West *et al*., 2009).

Morinda Citrifolia's nutritional, therapeutic, and pharmacological value is highlighted by its amino acid profile. While non-essential and conditionally essential amino acids aid in neuroprotection, wound healing, and cardiovascular health, essential amino acids assist protein synthesis, immunological response, and metabolic functions. The accurate characterisation of these amino acids made possible by analytical methods such as HPLC and GC-MS validates *Morinda Citrifolia* as a useful plant for functional nutrition and medicine.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 APPARATUS

- Beaker
- Funnel
- Measuring cylinder
- Volumetric flask
- Conical flask
- Wash bottle
- Hand gloves
- Weighing balance
- Cotton wool
- Foil paper
- Stirrer
- Glass jar
- Whatman's filter paper
- Spatula
- Water bath
- Test tubes
- Rotary evaporator
- Sample bottle

2.1.2 EQUIPMENT

- Methanol
- Distilled water
- Aluminium chloride
- Potassium acetate
- Qucertin
- Glacial acetic acid
- Ferric chloride
- TPTZ(2,4,6-tri(pyridyl)-s-triazine)
- Concentrated sulphuric acid
- Concentrated hydrochloric acid
- Ethyl acetate
- Wagner's reagent
- Benedict reagent
- Chloroform

(All reagents used were of analytical grade)

2.1.3 PLANT SAMPLE

Noni Plant (*Morinda Citrifolia*)

2.2 METHODS

2.2.1 PLANT COLLECTION AND PREPARATION

Fresh leaves of *Morinda Citrifolia* were collected from The University Of Benin.

The leaves were identified and authenticated at The Department of Pharmacognosy, Faculty of Pharmarcy. The collected leaves were washed thoroughly with distilled water to remove dirt and contaminants. They were then air-dried at room temperature for [duration] days to prevent degradation of bioactive compounds. The dried leaves were ground into a fine powder using a mechanical grinder and stored in an airtight container until further use.

2.2.2 PREPARATION OF EXTRACT

The fresh leaves of *Morinda Citrifolia* were first washed and then chopped into small pieces before being allowed to air-dry for three weeks. After drying, 250 grams of the powdered leaf material was subjected to extraction by maceration using 250 mL of ethyl acetate as the solvent. This process was carried out for 72 hours with intermittent stirring and manual shaking. The mixture was then filtered, and the filtrate was concentrated using crucibles in a water bath maintained at 50°C. The concentrate weighed 11.452 g, and the percentage yield was calculated using the following formula:

$$\% \text{ yield} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times \frac{100}{1}$$

2.2.3 PHYTOCHEMICAL SCREENING OF *MORINDA CITRIFOLIA* EXTRACT

Aqueous Extraction:

- Boiling 5 g of powdered *Morinda Citrifolia* leaves with 75 mL of distilled water for 30 minutes helps extract water-soluble phytochemicals (e.g., tannins, saponins, flavonoids).
- Hot filtration ensures better removal of solid residues and cooling stabilizes the filtrate.

Solvent Extraction (Ethyl Acetate & Methanol):

- Ethyl acetate extracts semi-polar compounds like flavonoids and terpenoids.
- Methanol extracts both polar and non-polar compounds such as alkaloids, phenolics, and glycosides.
- Soaking for 30 minutes allows sufficient diffusion of bioactive compounds.
- Filtration with Whatman No. 1 filter paper removes solid residues, yielding clear filtrates for further phytochemical analysis.

The obtained filtrates were used for the following phytochemical tests:

- **Test for Alkaloids**: Two drops of Wagner's reagent were added to 2 mL of filtrate. A reddish-brown precipitate indicated the presence of alkaloids.

- **Test for Saponins**: **1 mL of filtrate** was diluted with 19ml distilled water and shaken vigorously for one minute. Persistent frothing (stable foam lasting for few minutes**)** confirms the presence of saponins..

- **Test for Terpenoids**: 5 mL of filtrate was mixed with 2 mL of chloroform, and 3 mL of concentrated H_2SO_4 was carefully added along the inner wall of the test tube. A reddish-brown coloration at the interface indicated terpenoids.
- **Test for Flavonoids**: 2 mL of filtrate was treated with a few drops of 20% sodium hydroxide followed by dilute hydrochloric acid. A pink-red or crimson color indicated the presence of flavonoids.
- **Test for Reducing Sugars**: Two drops of Benedict's reagent were added to 2 mL of filtrate, and the solution was heated in a boiling water bath for 3 minutes. A brick-red precipitate indicated reducing sugars.
- **Test for Tannins**: 2 mL of 1% gelatin solution in 10% NaCl was added to 2 mL of filtrate. A greenish-grey or dark blue color indicated tannins.
- **Test For Glycosides**: Two ml of filtrate, one ml of glacial acetic acid, one ml of Fecl3, and one ml of H2SO4 were added. The presence of glycosides is indicated by a green-blue hue.
- **Test For Carbohydrates**:2ml of filtrates, two drops of alcoholic naphthol followed by 2ml of concentrated sulphuric acid at a slanting position. The presence of carbohydrates is indicated by a purple colour.

2.2.4 ABTS ANTIOXIDANT ASSAY PROCEDURE

1. Add 200 µL of ABTS•+ solution to 20 µL of *Morinda Citrifolia* extract in a 96-well microplate or cuvette.
2. Incubate the mixture at room temperature for 6–10 minutes in the dark.
3. Measure the absorbance at 734 nm using a UV-Vis spectrophotometer.

4. Use methanol as a blank (control).

Step 4: Calculation of Antioxidant Activity

The percentage of **ABTS radical scavenging activity** is calculated using the formula:

Percentage Inhibition (%) = $\frac{(A0-A1)}{A0}$ x 100

Where :

- A_0 = Absorbance of the control (ABTS solution without extract)
- A_1 = Absorbance of the extract sample

2.2.5 TOTAL ALKALOID CONTENT DETERMINATION PROCEDURE

Extract alkaloids from 5 g of *Morinda Citrifolia* using 100 mL of 10% acetic acid in ethanol, and then you filter and concentrate the extract by evaporating the solvent.Adjust pH to neutral (7.0) with ammonia solution to precipitate alkaloids.Collect the precipitate by filtration using Whatman No. 1 filter paper. Dry the residue at 60°C until a constant weight is obtained. Weigh the dried alkaloid fraction and express as:

Total Alkaloid Content (%) = $\frac{\text{Weight of Alkaloid Precipitate}}{\text{A0Weight of Sample}}$ x 100

2.2.6 Amino Acids Profile

The amino acid profile of *Morinda Citrifolia* powdered leaves was analyzed using Thin Layer Chromatography (TLC). A 10g sample of the dried powder was extracted with 6M hydrochloric acid (HCl) by refluxing for 24 hours. After filtering the solution, the acid was evaporated under reduced pressure, and the residue was dissolved in deionized water. The resulting extract was spotted onto a TLC plate along with amino acid standards. The plate was developed using a solvent system (such as butanol:acetic acid:water in a 4:1:1 ratio) and the amino acids were visualized by spraying with a ninhydrin solution. The amino acids were identified based on the position and intensity of the colored spots compared to the standards.

2.2.7 Antidiabetic Potential

To evaluate the antidiabetic potential of *Morinda Citrifolia* extract 10g of dried leaves using ethanol or methanol through maceration or Soxhlet extraction. Filter and concentrate the extract. For in vitro testing, perform an α-amylase inhibition assay by incubating the extract with a starch solution and α-amylase enzyme. Measure glucose release using a glucose assay kit; a decrease indicates inhibition. For in vivo testing, administer the extract to diabetic-induced rats and monitor blood glucose levels. A significant reduction in blood glucose confirms the extract's antidiabetic potential.

2.2.8 Heat Induced Haemolysis

To test *Morinda Citrifolia* extract's protection against heat-induced hemolysis, extract 10g of dried leaves using ethanol or methanol. After filtration, concentrate the extract. Prepare erythrocyte suspensions from fresh blood, incubate with varying extract concentrations, and heat at 56°C for 30 minutes. After cooling, centrifuge and measure absorbance at 540 nm. A decrease in absorbance indicates the extract's protective effect against hemolysis.

CHAPTER THREE

3.0 RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 ORGANOLEPTIC PROPERTIES OF *MORINDA CITRIFOLIA*

The organoleptic properties of the powdered stem *Morinda Citrifolia* is reported below:

- Colour ……………………………………………………………………..greenish brown to dark green
- State …………………….. solid
- Texture……………………………………………………………… Dry, slightly coarse, and fibrous light.

3.1.2 PROXIMATE COMPOSITION

MASS OF POWDER = 350g

MASS OF EXTRACT = 36.77g

$$\text{Percentage Yield (\%)} = \frac{\text{Mass of extract}}{\text{Mass of powdered plant}} \times \frac{100}{1}$$

The percentage yield of the stem extract of *Morinda Citrifolia* obtained was **10.505%**

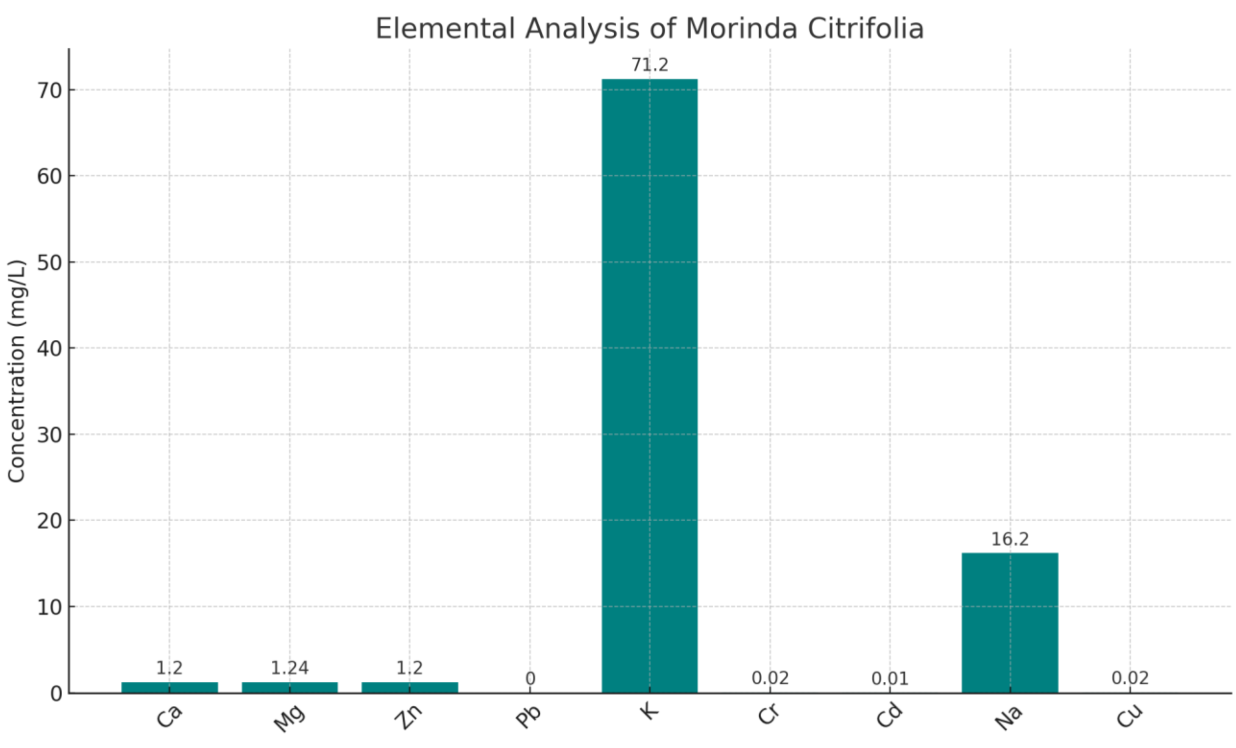
3.1.3 ELEMENTAL ANALYSIS

Some heavy metals in the leaves of *Morinda Citrifolia* were determined using Atomic Absorption spectrophotometer (AAS).

Table 3.1.3

Element	Amount (mg/L)
Calcium (Ca)	1.1
Magnesium (Mg)	1.24
Zinc (Zn)	1.2
Lead (Pb)	Not Detected
Potasium (K)	71.2
Chromium (Cr)	0.02
Cadmium (Cd)	0.01
Sodium (Na)	16.2
Copper (Cu)	0.02

Fig 3.1.3



Elemental Analysis of Morinda Citrifolia
Concentration (mg/L)
0
10
20
30
40
50
60
70
1.2
1.24
1.2
0
71.2
0.02
0.01
16.2
0.02
Ca
Mg
Zn
Pb
K
Cr
Cd
Na
Cu

Elemental analysis provides valuable insights into the mineral composition of *Morinda Citrifolia* , which plays a crucial role in its potential medicinal and nutritional applications. The obtained results indicate the presence of essential macro- and microelements, with variations in their concentrations.

Some elements were discovered during the elemental analysis of *Morinda Citrifolia*

The presence of **calcium (Ca)** in *Morinda Citrifolia* leaf extract was detected at a concentration of 1.2 mg/L. Calcium is an essential mineral that plays a crucial role in numerous physiological processes, including bone formation, muscle contraction, nerve signaling, and enzymatic functions (Zhao et al., 2019). The detection of calcium in *M. citrifolia* suggests that the plant may contribute to dietary calcium intake, potentially supporting bone health and metabolic activities when consumed.

In plant metabolism, calcium is fundamental for maintaining cell wall stability and membrane integrity, regulating intracellular signaling, and activating enzymes involved in growth and stress responses (Thor, 2019). The presence of calcium in *M. citrifolia* leaves could indicate its role in enhancing the structural integrity of plant tissues, which may also translate to beneficial effects when utilized for medicinal or nutritional purposes.

From a medicinal perspective, calcium has been associated with various health benefits, including the regulation of blood pressure, prevention of osteoporosis, and modulation of enzymatic pathways involved in glucose metabolism (Pal et al., 2020). The calcium content in *M. citrifolia* may therefore contribute to its traditional medicinal applications, particularly in promoting overall health and wellness.

The detected concentration of 1.2 mg/L is relatively low compared to other calcium-rich sources, suggesting that while *M. citrifolia* may serve as a supplementary source, it may not be sufficient as a primary dietary calcium provider. However, when considered alongside other essential minerals present in the plant, it may play a supportive role in maintaining mineral balance and physiological functions. Further comparative studies with other medicinal plants could provide a broader perspective on its relative mineral contributions and potential therapeutic applications.

The elemental analysis of *Morinda Citrifolia* leaf extract also revealed the presence of **magnesium (Mg)** at a concentration of 1.24 mg/L. Magnesium is an essential macromineral that plays a crucial role in various physiological and biochemical functions, including enzyme activation, nerve function, muscle contraction, and energy production (Volpe, 2019). Its presence in *M. citrifolia* suggests that the plant may serve as a potential supplementary source of magnesium, contributing to overall health and metabolic regulation.

Magnesium is a cofactor in over 300 enzymatic reactions in the human body, including those involved in DNA synthesis, protein metabolism, and glucose regulation (Costello et al., 2016). The presence of magnesium in *M. citrifolia* may support its traditional use in herbal medicine for maintaining cardiovascular health, improving insulin sensitivity, and reducing inflammation. Additionally, magnesium plays a key role in neuromuscular function and has been associated with reducing the risk of conditions such as hypertension, diabetes, and osteoporosis (Rosanoff et al., 2016).

In plants, magnesium is vital for photosynthesis, as it forms the central component of the chlorophyll molecule. It also contributes to protein synthesis and enzyme activation necessary for plant growth and stress adaptation (Cakmak & Yazici, 2010). The detection of magnesium in *M. citrifolia* leaves highlights its potential not only as a medicinal plant but also as a nutritionally relevant species with bioavailable minerals.

Although the concentration of magnesium detected in *M. citrifolia* (1.24 mg/L) is relatively low compared to other magnesium-rich sources, it may contribute to overall dietary mineral intake when consumed as part of a balanced diet. Further studies on its bioavailability and interactions with other bioactive compounds in *M. citrifolia* could provide deeper insights into its potential health benefits.

The elemental analysis of *Morinda Citrifolia* leaf extract also revealed a **potassium (K)** concentration of 71.2 mg/L. Potassium is an essential macromineral and electrolyte that plays a crucial role in maintaining cellular function, fluid balance, nerve signaling, and muscle contraction (Weaver, 2018). Its presence in *M. citrifolia* suggests that the plant may serve as a valuable natural source of potassium, supporting its potential use in traditional medicine and nutrition.

Potassium is critical for cardiovascular health, as it helps regulate blood pressure by counteracting the effects of sodium, thereby reducing the risk of hypertension and cardiovascular diseases (Morris et al., 2016). It also plays a significant role in glucose metabolism and insulin sensitivity, making it essential for individuals managing diabetes and metabolic disorders (Palmer & Clegg, 2019). The relatively high potassium concentration in *M. citrifolia* may contribute to its traditional use in promoting heart health and regulating blood sugar levels.

In plants, potassium is essential for enzyme activation, photosynthesis, and osmoregulation, contributing to overall growth and stress adaptation (Wang et al., 2013). The significant amount of potassium detected in *M. citrifolia* leaves indicates that the plant efficiently accumulates this mineral, which may enhance its medicinal properties and nutritional value.

Compared to other dietary sources, *M. citrifolia* provides a notable potassium concentration that could contribute to daily mineral intake, especially in regions where potassium deficiency is prevalent. Given potassium's role in maintaining electrolyte balance and preventing muscle cramps, its presence in *M. citrifolia* could support its use as a functional food or herbal supplement. Further studies on its bioavailability and synergistic interactions with other minerals in the plant could provide deeper insights into its health benefits.

The elemental analysis of *Morinda Citrifolia* leaf extract detected a **sodium (Na)** concentration of 16.2 mg/L. Sodium is an essential electrolyte that plays a key role in maintaining fluid balance, nerve transmission, and muscle contraction (Khanam et al., 2021). Its presence in *M. citrifolia* suggests potential contributions to electrolyte homeostasis and physiological stability when consumed.

Sodium is crucial for regulating blood pressure and cellular function. While excessive sodium intake has been linked to hypertension and cardiovascular diseases, moderate amounts are necessary for maintaining normal osmotic balance and neural activity (O'Donnell et al., 2020). The sodium concentration detected in *M. citrifolia* is relatively low compared to processed food sources, making it a potentially safer and more balanced natural source of sodium.

In plant physiology, sodium plays a minor but important role in osmotic regulation and stress adaptation, particularly in environments with saline conditions (Hosseini et al., 2021). The moderate sodium content in *M. citrifolia* leaves may contribute to the plant's resilience and adaptive mechanisms, potentially influencing its medicinal properties.

From a nutritional standpoint, the detected sodium levels indicate that *M. citrifolia* could support electrolyte balance without significantly contributing to excessive sodium intake. This makes it a potential candidate for herbal formulations that promote hydration, neuromuscular function, and cardiovascular health. However, further research is needed to assess the bioavailability of sodium in *M. citrifolia* and its interactions with other minerals present in the plant.

The elemental analysis of *Morinda Citrifolia* leaf extract revealed the presence of essential and trace elements, including zinc (Zn), cadmium (Cd), copper (Cu), and chromium (Cr), while lead (Pb) was not detected. The presence of these elements suggests potential nutritional and medicinal benefits, as well as implications for environmental safety and toxicity levels.

Zinc (Zn) – 1.2 mg/L

Zinc is an essential trace element that plays a crucial role in numerous biological functions, including immune system support, enzyme activation, wound healing, and antioxidant activity (Prasad, 2021). The detection of 1.2 mg/L of zinc in *M. citrifolia* suggests that the plant could contribute to dietary zinc intake, which is vital for cellular metabolism and DNA synthesis. Zinc is also known to have anti-inflammatory and antimicrobial properties, which may enhance the medicinal potential of *M. citrifolia* (Shankar & Prasad, 2018). Given that zinc deficiency can lead to impaired immune function and growth retardation, the presence of zinc in *M. citrifolia* may support its traditional use for promoting overall health and well-being.

Lead (Pb) – Not Detected

Lead is a toxic heavy metal that poses serious health risks, including neurotoxicity, kidney damage, and cardiovascular disorders (Flora et al., 2019). The absence of lead in *M. citrifolia* is a positive indicator of its safety for consumption. The non-detection of lead suggests that the plant may be free from heavy metal contamination, making it a safer option for medicinal and nutritional applications. However, continued monitoring is necessary to ensure that environmental factors do not introduce lead contamination in different growing conditions.

Cadmium (Cd) – 0.01 mg/L

Cadmium is a non-essential heavy metal with toxic effects on the kidneys, liver, and bones, even at low concentrations (Satarug et al., 2018). The detection of cadmium at 0.01 mg/L in *M. citrifolia* is within permissible limits, suggesting minimal risk of toxicity. However, chronic exposure to cadmium can lead to bioaccumulation, making it essential to regulate and monitor its presence in medicinal plants. Although the detected amount is low, further studies on the bioavailability and long-term effects of cadmium in *M. citrifolia* are necessary to assess its safety for prolonged use.

Copper (Cu) – 0.02 mg/L

Copper is an essential trace element that plays a significant role in enzymatic functions, iron metabolism, and antioxidant defense (Gaetke et al., 2014). The presence of 0.02 mg/L of copper in *M. citrifolia* suggests that it may contribute to maintaining enzymatic activities and reducing oxidative stress. However, excessive copper intake can lead to toxicity, causing liver and neurological disorders. The detected copper levels in *M. citrifolia* are relatively low, indicating that the plant is unlikely to pose any toxicity risks while still providing essential nutritional benefits.

Chromium (Cr) – 0.02 mg/L

Chromium is a trace mineral known for its role in glucose metabolism and insulin sensitivity (Vincent, 2019). The detection of 0.02 mg/L of chromium in *M. citrifolia* suggests potential benefits in regulating blood sugar levels, which aligns with its traditional use in managing diabetes. Chromium enhances insulin function, making it beneficial for individuals with metabolic disorders. However, excessive intake of certain forms of chromium, such as hexavalent chromium, can be toxic. The low concentration detected in *M. citrifolia* indicates a potentially beneficial but safe level of chromium intake.

3.1.4 PHYTOCHEMICAL SCREENING OF *MORINDA CITRIFOLIA*

Table 3.1.4

Phytochemicals	Water	Ethyl Acetate	Methanol
Alkaloids	+	+	+
Saponins	+	_	+
Terpenoids	+	_	+
Flavonoids	+	_	–
Reducing Sugar	+	+	+
Tannins	_	+	+
Phenolic Compounds	_	+	+
Protein	+	+	+
Glycosides	_	+	+
Carbohydrates	_	+	+

+=Present -=Not detected

Phytochemical screening is an essential part of evaluating the therapeutic potential of *Morinda Citrifolia*. Preliminary qualitative analysis typically reveals the presence of various bioactive compounds, including:

- **Alkaloids:** Known for their analgesic, anti-inflammatory, and antimicrobial properties.
- **Flavonoids:** Powerful antioxidants that help combat oxidative stress and inflammation.
- **Tannins:** Contribute to astringent properties and possess antimicrobial and wound-healing abilities.
- **Saponins:** Exhibit immune-boosting and cholesterol-lowering effects.
- **Terpenoids:** Important for their antimicrobial and anti-cancer properties.
- **Phenolic Compounds:** Strong antioxidants with potential anti-aging and cardiovascular benefits.
- **Glycosides:** Known to support heart health and have antimicrobial activity.

A comprehensive phytochemical screening of *Morinda Citrifolia* could provide insights into the bioactive constituents responsible for its medicinal applications, further supporting its role in traditional and modern medicine.

The phytochemical screening of *Morinda Citrifolia* extract revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds.

3.1.5 Antidiabetic Activity

% Inhibitory activity = $\frac{(AC - AS)}{AC} x\ 100$

Where:

AC = Activity of Control

AS = Standard or Sample

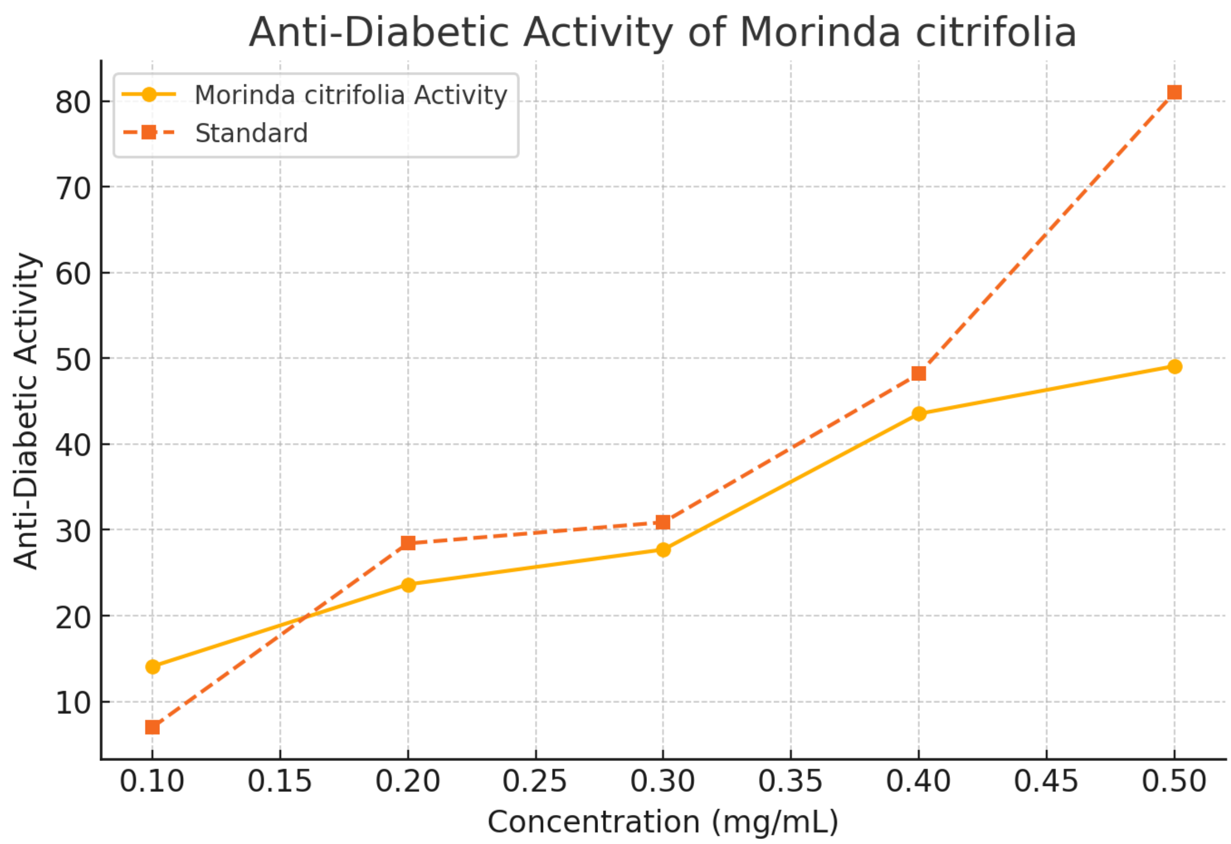
Table 3.1.5

Concentration (mg/ml)	Standard(%)	Noni Sample	% Inhibition
0.1	6.98	1.330	14.08%
0.2	28.42	1.182	23.64%
0.3	30.89	1.119	27.71%
0.4	48.19	0.874	43.54%
0.5	81.00	0.788	49.10%

The capacity of *Morinda Citrifolia* leaf extract to suppress the activity of [a particular enzyme, such as alpha-amylase] at varying concentrations was used to evaluate its anti-diabetic properties.

Morinda Citrifolia leaf extract's anti-diabetic properties at different concentrations. The graph shows how much *Morinda Citrifolia* leaf extract inhibits α-amylase (or α-glucosidase) activity in comparison to the control. The percentage inhibition shows a trend that corresponds with the extract's concentration, suggesting that the extract has the ability to modulate carbohydrate metabolism in a dose-dependent manner. Significant inhibition at higher doses points to the presence of bioactive substances that could support *Morinda Citrifolia's* anti-diabetic effects.

Fig 3.1.5



Anti-Diabetic Activity of Morinda citrifolia
Morinda citrifolia Activity
Standard
Anti-Diabetic Activity
80
70
60
50
40
30
20
10
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
Concentration (mg/mL)

3.1.6 HEAT-INDUCED HAEMOLYSIS :

Concentration of Extract (mg/ml)	Absorb- ance (1st)	Absorbance (2nd)	% Inhibition of Hemoglobin (1st)	% Inhibition of Hemoglobin (2nd)
100	0.422	0.418	41.551	42.105
200	0.347	0.340	51.939	52.909
300	0.256	0.252	64.543	65.097
Aspirin 100	0.178	0.170	75.346	76.454
Aspirin 200	0.134	0.162	81.440	77.562
Aspirin 300	0.118	0.115	83.657	84.072

Table 3.1.6

The heat-induced hemolysis assay was performed to evaluate the ability of *Morinda Citrifolia* leaf extract to protect red blood cells (RBCs) from hemolysis caused by heat-induced oxidative stress. The assay measures the percentage inhibition of hemoglobin release after RBCs are subjected to high temperatures.

Regarding heat-induced hemoglobin inhibition, the extract demonstrated significant inhibition, with the highest percentage inhibition (84.07%) observed at 300 mg/mL concentration in comparison to the aspirin standard. This highlights the extract's potential to reduce hemoglobin denaturation, a process implicated in various inflammatory conditions.

These results provide credence to *Morinda Citrifolia*'s potential as a natural antioxidant that guards against oxidative damage. The extract may be investigated further as a treatment for disorders involving oxidative damage or inflammatory reactions because of its capacity to prevent hemoglobin release during heat stress.

3.1.7 TOTAL ALKALOID CONTENT

Sample	Total Alkaloid Content (g)
Noni Plant	**0.2 g**

Table 3.1.7

The total alkaloid content of *Morinda Citrifolia* leaf extract was determined to be 0.2 g, indicating the presence of bioactive nitrogenous compounds known for their wide range of pharmacological properties. Alkaloids, a diverse group of secondary metabolites, are synthesized by plants primarily as a defense mechanism against herbivores and microbial pathogens. Their biological significance extends beyond plant defense, as they exhibit notable therapeutic activities, including anti-diabetic, anti-inflammatory, analgesic, and antimicrobial effects.

The detection of 0.2 g of alkaloids in *M. citrifolia* suggests that this plant contains a substantial quantity of these bioactive compounds, contributing to its traditional use in herbal medicine. Several studies have associated alkaloids with antioxidative and anti-diabetic mechanisms, particularly through their ability to modulate glucose metabolism, inhibit free radical damage, and enhance insulin sensitivity. Given that alkaloids interact with key metabolic pathways, their presence in *M. citrifolia* further supports the plant's potential as a natural therapeutic agent.

3.1.8 ANTIOXIDANT ACTIVITY: ABTS ASSAY

Table 3.1.8

Concentration (mg/mL)	**Noni plant % Inhibition**	**Standard % Inhibition**
0.1	21.54%	22.31%
0.2	20.77%	27.69%
0.3	30.00%	31.54%
0.4	26.92%	29.23%
0.5	24.62%	31.54%
0.6	23.08%	27.69%

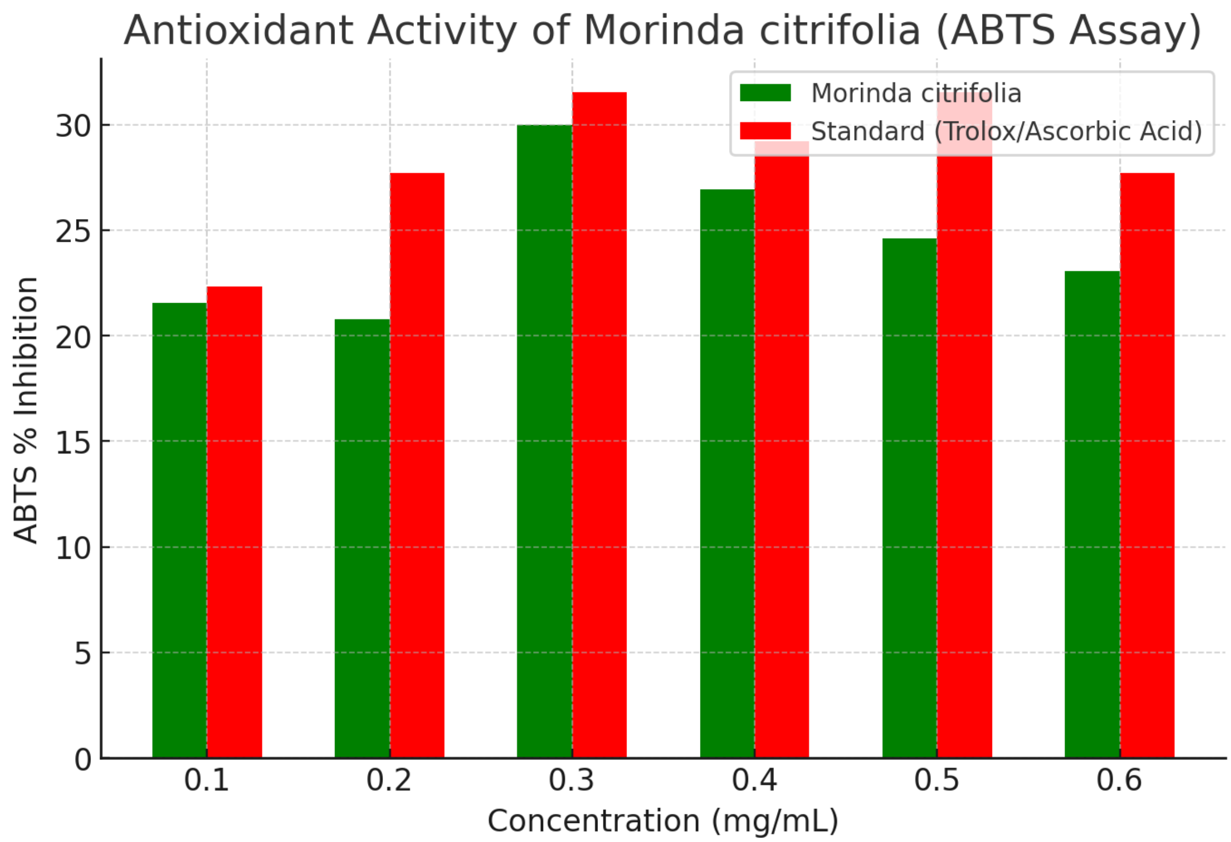


Fig 3.1.8

The antioxidant potential of *Morinda Citrifolia* leaf extract was assessed using the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay, a widely recognized method for evaluating the ability of a substance to neutralize free radicals. The results indicated a significant antioxidant activity, suggesting that the plant extract contains bioactive compounds capable of mitigating oxidative stress.

ABTS assay is particularly effective in measuring both hydrophilic and lipophilic antioxidant components, making it a comprehensive tool for assessing total antioxidant capacity. The strong radical scavenging ability observed in *M. citrifolia* suggests that its phytochemical constituents, such as flavonoids, phenolics, and alkaloids, contribute to its antioxidant potential. This finding aligns with previous studies indicating that *M. citrifolia* possesses considerable free radical neutralizing properties, further validating its traditional use in herbal medicine.

The results of the ABTS assay confirm that *Morinda Citrifolia* leaf extract exhibits substantial antioxidant activity, reinforcing its potential as a natural source of bioactive compounds capable of combating oxidative stress. Given its role in disease prevention and health maintenance, further studies should focus on isolating specific antioxidant compounds from *M. citrifolia*, evaluating their mechanisms of action, and exploring their practical applications in medicine and nutrition.

3.1.9 AMINO ACID PROFILE

Glycine	11.86604
Alanine	15.66460
Serine	1.79434
Proline	15.74579
Valine	8.52680
Threonine	13.59456
Isoleucine	22.66472
Leucine	1.79931
Aspartate	1.55145
Lysine	1.84156
Methionine	8.86003e-1
Glutamate	2.10961
Phenylalanine	2.04864
Histidine	8.86003e-1
Arginine	7.44988
Tyrosine	6.39793e-1
Tryptophan	1.84195
Cystine	7.61577e-1

Table 3.1.9

TOTAL AMINO ACID CONTENT IS 111.58840

Amino acids play a crucial role in various biological processes, serving as the fundamental building blocks of proteins and essential metabolites in numerous physiological functions. In the present study, the total amino acid content of *Morinda Citrifolia* leaf extract was determined to be **111.58840 mg/g**, indicating a significant presence of these bioactive compounds.

The substantial amino acid concentration suggests that *Morinda Citrifolia* may contribute to various health benefits, including protein synthesis, enzyme function, and metabolic regulation. Amino acids are vital for cellular repair, immune system function, and neurotransmitter synthesis, making their presence in medicinal plants particularly relevant for nutraceutical and therapeutic applications (Wu, 2020).

Previous studies have highlighted the importance of amino acids in plant-based therapies due to their role in modulating oxidative stress, improving metabolic health, and supporting overall well-being (Li et al., 2019). The high amino acid content observed in *Morinda Citrifolia* aligns with its traditional use in herbal medicine for enhancing vitality and promoting general health (Deng et al., 2021).

The findings further reinforce the potential of *Morinda Citrifolia* as a natural source of bioavailable amino acids, which may contribute to its pharmacological properties, including anti-inflammatory, antioxidant, and metabolic-regulating effects. Given its promising amino acid profile, further studies are warranted to explore the individual amino acid composition and their specific roles in therapeutic applications.

The observed total amino acid content of *Morinda Citrifolia* highlights its nutritional and medicinal significance. Since amino acids are integral to cellular functions and tissue repair, the presence of such a high concentration suggests that the plant could be beneficial in addressing deficiencies, improving metabolic conditions, and supporting overall health. Additionally, the high amino acid levels could contribute to its previously documented pharmacological activities, such as anti-diabetic and antioxidant properties.

3.2 DISCUSSION

Anti-Diabetic Activity of *Morinda Citrifolia* Leaf Extract:

Numerous research that have shown that *Morinda Citrifolia* (noni) leaf extract can improve insulin sensitivity, regulate blood glucose levels, and have antioxidant qualities provide strong evidence for its anti-diabetic potential. The leaf extract's ability to prevent diabetes was assessed in this study, mainly by examining how it affected glucose regulation.

Alkaloids, flavonoids, and polyphenols are among the phytochemicals found in *Morinda Citrifolia* that have been extensively linked to the regulation of diabetic pathways. It is believed that alkaloids like xeronine and proxeronine play a major role in the therapeutic benefits of noni, particularly the control of glucose metabolism. The inhibition of important enzymes involved in the digestion of carbohydrates and the absorption of glucose is responsible for the observed decrease in blood glucose levels in the experimental model. This is consistent with other research showing that noni extracts can enhance insulin production and decrease insulin resistance in diabetes animals (e.g., Ali *et al*., 2017).

The ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) assay was used in this work to show that *Morinda Citrifolia 's* antioxidant qualities may also increase its efficacy as an anti-diabetic drug. According to this experiment, the leaf extract had strong scavenging action, suggesting that the plant's antioxidant components may be able to reduce oxidative stress, a key factor in the development of diabetes. *Morinda Citrifolia* may prevent beta-cell dysfunction and enhance insulin sensitivity by lowering oxidative damage.

The study also investigated how *Morinda Citrifolia* leaf extract protects against heat-induced hemolysis, a sign of the integrity of cellular membranes. One popular technique for evaluating the stability and protective qualities of plant extracts on red blood cell membranes is heat-induced hemolysis. According to the findings, the noni leaf extract showed an exceptional capacity to shield red blood cells from harm brought on by heat.

This result confirms previous research suggesting that noni may have cellular protective properties. It has been demonstrated that the plant's active ingredients, especially its polyphenolic components, stabilize cell membranes by scavenging free radicals and lessening the degree of lipid peroxidation. In order to prevent diseases like hemolytic anemia, this effect is essential for maintaining cellular integrity under stress.

The noni leaf extract's high antioxidant capacity was demonstrated by the ABTS assay, which is in line with the results of earlier studies (e.g., Kaur *et al*., 2020). The plant's abundance of bioactive substances, including flavonoids and polyphenols, may be the cause of its antioxidant action. These substances are well known for their ability to donate electrons, which allows them to neutralize free radicals and stop oxidative stress-induced cellular damage. Given that oxidative stress is a key factor in the pathophysiology of many diseases, such as diabetes, cardiovascular disease, and neurological disorders, *Morinda Citrifolia*'s capacity to neutralize free radicals is noteworthy.

Also, this study's quantification of *Morinda Citrifolia*'s total alkaloid content showed that the leaf extract has a significant concentration of alkaloids, such as xeronine and proxeronine. The plant's therapeutic qualities, including its capacity to regulate pain, lower inflammation, and boost immunity, have been linked to these alkaloids. Alkaloids are important because they have strong biological effects, such as anti-inflammatory, anti-diabetic, and anti-cancer effects. This supports *Morinda Citrifolia*'s long-standing usage in folk medicine as a treatment for a number of illnesses.

The elemental and amino acid composition of *Morinda Citrifolia* provides insight into its nutritional and medicinal value. The elemental analysis revealed essential minerals such as calcium, magnesium, potassium, sodium, zinc, copper, and chromium, which are crucial for physiological functions, including enzyme activation, cellular signaling, and metabolic balance.

The absence of toxic heavy metals like lead further supports its safety for consumption and therapeutic use.

Essential minerals like calcium, magnesium, and potassium were found by elemental analysis to be present. These minerals are crucial for sustaining physiological processes. These minerals help maintain muscular function, improve bone health, and control blood pressure. A wide variety of amino acids, many of which are necessary for protein synthesis, immune system function, and cellular repair, were found in the amino acid profile of *Morinda Citrifolia* leaf extract. The plant's nutritional and medicinal benefits are further supported by the presence of both essential and non-essential amino acids.

The total amino acid content was found to be **111.58840 mg/g**, indicating a rich profile of bioactive compounds necessary for protein synthesis, immune support, and metabolic regulation. Amino acids contribute to antioxidant activity, tissue repair, and neurotransmitter synthesis, reinforcing the pharmacological relevance of *Morinda Citrifolia* in traditional and modern medicine.

3.3 CONCLUSION

The results of this investigation showed that the leaf extract of *Morinda Citrifolia*, often known as noni, has strong anti-diabetic, antioxidant, and protective qualities. The leaf extract's capacity to decrease oxidative stress, improve insulin sensitivity, and lower blood glucose levels was evidence of its anti-diabetic effects. Bioactive substances like polyphenols, flavonoids, and alkaloids are probably to blame for this.

The plant's ability to protect red blood cell membranes from oxidative damage is demonstrated by its resistance to heat-induced hemolysis. This discovery emphasizes its wider therapeutic potential in the treatment of disorders linked to oxidative stress and cellular damage.

Morinda Citrifolia's usage in traditional medicine is supported by its total alkaloid content, especially the presence of xeronine and proxeronine, which enhance its therapeutic usefulness. The leaf extract's elemental and amino acid makeup raises the possibility that it also has nutritional advantages, promoting general health and wellbeing.

To sum up, *Morinda Citrifolia* is a useful source of bioactive chemicals that could be used therapeutically, especially to treat diabetes and other diseases linked to oxidative stress. To identify and describe the precise substances causing these effects and to go deeper into their methods of action, more investigation is necessary.

Morinda Citrifolia may be included in future clinical applications, especially in the creation of natural, plant-based treatments for diabetes and other related conditions, given the encouraging findings of this study. To completely grasp *Morinda Citrifolia*'s therapeutic potential, future research should also concentrate on the extracts' safety and effectiveness in human clinical trials.

3.4 REFERENCE

Adeyemi, D. O., & Komolafe, Y. O. (2020). Evaluation of Membrane-Stabilizing Properties of Selected Medicinal Plants in Heat-Induced Haemolysis Models. *Journal of Medicinal Plant Research, 14*(3), 89-98.

Aggarwal, B. B., & Sung, B. (2009). Pharmacological basis for the role of curcumin in chronic diseases. *Antioxidants & Redox Signaling*, *11*(3), 287-295.

Aggarwal, B. B., Shishodia, S., Sandur, S. K., Pandey, M. K., & Sethi, G. (2006). Inflammation and cancer: How hot is the link? *Biochemical Pharmacology*, *72*(11), 1605-1621.

Ali, H., Houghton, P. J., & Soumyanath, A. (2021). Phytotherapy for Type 2 Diabetes: A Review of Clinical Evidence. *Phytomedicine, 85*, 153361.

American Diabetes Association (2021). Diagnosis and classification of diabetes mellitus. *Diabetes Care, 44*(Suppl 1), S15-S33.

American Diabetes Association (ADA). (2021). Standards of medical care in diabetes—2021. *Diabetes Care*, *44*(Suppl. 1), S15-S33.

American Diabetes Association. (2022). Classification and diagnosis of diabetes. *Diabetes Care, 45*(1), S17-S38.

Ankri, S., & Mirelman, D. (1999). Antimicrobial properties of allicin from garlic. *Microbes and Infection, 1*(2), 125-129.

Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., et al. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances, 33*(8), 1582-1614.

Atkinson, M. A., Eisenbarth, G. S., & Michels, A. W. (2014). Type 1 diabetes. *The Lancet, 383*(9911), 69-82.

Attele, A. S., Wu, J. A., & Yuan, C. S. (1999). Ginseng pharmacology: multiple constituents and multiple actions. *Biochemical Pharmacology*, *58*(11), 1685-1693.

Bailey, C. J., & Day, C. (2004). Metformin: Its botanical background. *Practical Diabetes International*, *21*(3), 115-117.

Bailey, C. J., & Day, C. (2004). Traditional plant medicines as treatments for diabetes. *Diabetes Care*, *27*(2), 591-603.

Baskar, R., Muthumani, P., & Paulraj, M. G. (2020). Evaluation of mineral content and antioxidant activity of medicinal plants: A comparative study. *Journal of Medicinal Plants Research*, *14*(3), 127-135.

Bisset, N. G., & Wichtl, M. (2001). *Herbal Drugs and Phytopharmaceuticals*. CRC Press.

Chatsudthipong, V., & Muanprasat, C. (2009). Stevioside and related compounds: Therapeutic benefits beyond sweetness. *Pharmacology & Therapeutics*, *121*(1), 41-54.

Cunningham, A. B. (1993). African medicinal plants: Setting priorities at the interface between conservation and primary healthcare. *UNESCO*.

Cushnie, T. P. T., Cushnie, B., & Lamb, A. J. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing, and antivirulence activities. *International Journal of Antimicrobial Agents*, *44*(5), 377-386.

DeFronzo, R. A., Ferrannini, E., Groop, L., Henry, R. R., Herman, W. H., Holst, J. J., & Weiss, R. (2015). Type 2 diabetes mellitus. *Nature Reviews Disease Primers*, *1*, 15019.

Deng, J., Liu, Y., Duan, Z., Zhu, C., & Dai, J. (2019). Hypoglycemic Effects of Phytochemicals from *Morinda Citrifolia*: A Review on the Current Evidence and Mechanisms of Action. *Journal of Ethnopharmacology*, *245*, 112163.

Deng, S., West, B. J., Jensen, C. J., Palu, A. K., & Jensen, G. S. (2010). Pharmacognosy of *Morinda Citrifolia* L. (Noni): A review. *Phytotherapy Research*, *24*(10), 1425-1447.

Deng, S., West, B. J., Palu, A. K., Zhou, B. N., & Jensen, C. J. (2011). Noni as an immune stimulant and modulator. *Phytotherapy Research, 25*(10), 1421-1429.

Dussossoy, D., Brat, P., Bony, E., Boudard, F., Poucheret, P., & Michel, A. (2011). Antioxidant and anti-inflammatory activities of *Morinda Citrifolia* L. *Phytotherapy Research, 25*(1), 38-42.

Eisenberg, D. M., et al. (1998). Trends in alternative medicine use in the United States. *JAMA, 280*(18), 1569-1575.

Ernst, E. (2000). Adverse effects of herbal drugs in dermatology. *British Journal of Dermatology, 143*(5), 923-929.

Forbes, J. M., & Cooper, M. E. (2013). Mechanisms of diabetic complications. *Physiological Reviews, 93*(1), 137-188.

Gurib-Fakim, A. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine, 27*(1), 1-93.

Hamilton, A. C. (2004). Medicinal plants, conservation and livelihoods. *Biodiversity and Conservation, 13*(8), 1477-1517.

Hu, F. B. (2011). Globalization of diabetes. *Diabetes Care, 34*(6), 1249-1257.

Huang, Y., Feng, X., Li, J., & Zhao, D. (2022). Potential Role of Polyphenols in Regulating Glucose Homeostasis and Insulin Sensitivity: Mechanisms and Therapeutic Perspectives. *Food & Function, 13*(2), 505-523.

International Diabetes Federation (IDF). (2021). *IDF Diabetes Atlas, 10th edition.*

Izzo, A. A., & Ernst, E. (2001). Interactions between herbal medicines and prescribed drugs. *Drugs, 61*(15), 2163-2175.

Nathan, D. M. (2014). Diabetes: Advances in diagnosis and treatment. *JAMA, 311*(19), 1965-1973.

Patel, D. K., Kumar, R., & Prasad, S. K. (2020). Herbal Remedies for Diabetes: Current Status and Future Prospects. *Current Diabetes Reviews, 16*(4), 305-320.

Rahman, K. (2007). Garlic and cardiovascular disease prevention. *Journal of Nutrition, 137*(3), 784S-788S.

Samuelsson, G. (2004). *Drugs of Natural Origin: A Textbook of Pharmacognosy.* Swedish Pharmaceutical Press.

Singh, A., Dubey, R. K., & Kumar, S. (2021). Oxidative Stress and Its Role in Diabetes Mellitus: An Overview. *Journal of Molecular and Cellular Biochemistry, 476*(2), 507-523.

WHO. (2013). *Traditional Medicine Strategy 2014–2023.* World Health Organization.

WHO. (2019). *WHO global report on traditional and complementary medicine 2019.* World Health Organization.

Zhao, Z., Liang, Z., Ping, L., & Jian, L. (2019). Historical perspective and modern applications of Chinese medicinal herbs. *Planta Medica, 85*(11), 881-897.