In-vitro Phytochemical and Antibacterial Comparative Evaluation of _Ocimum gratissimum_ and _Moringa Oleifera_ leaves on some Enteric Bacteria

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Certification

This is to certify that **Oluseyi Oluyemisi ELATUROTI** with matriculation number LCU/PG/000266 carried out this research work titled "***Invitro*** **Phytochemical and Antibacterial Comparative Evaluation of *Ocimum gratissimum* (Scent Leaves) and *Moringa Oleifera* leaf extracts on some Enteric Bacteria.**" in the Department of Biological Science, Faculty of Natural and Applied Sciences, Lead City University Ibadan, Nigeria for the award of Master Degree in Medical Microbiology and that this has not been previously submitted.

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Dedication

To God be the glory, honour and adoration, I dedicate this research to Almighty God from whom I received sufficient grace and strength for the successful completion of this project work.

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Even though the above mentioned institutions and persons have assisted in this work, I alone stand responsible for errors, if any, found in this work.

Abstract

The increasing resistance of some enteric bacteria to antibiotics has necessitated the need for non-conventional medicine against them. This study evaluated the phytochemical composition and antibacterial activity of *Ocimum gratissimum* (scent leaf) and *Moringa oleifera* leaf extracts against clinical isolates, comparing their efficacy with standard antibiotics. Phytochemical screening revealed the presence of phenols, alkaloids, glycosides, tannins, saponins, and terpenoids in varying concentrations, with methanol and aqueous extracts showing the highest yields. Quantitative analysis indicated that phenols and glycosides were predominant, particularly in methanol and aqueous extracts. The antibacterial activity of the extracts was tested on some enteric bacteria using different solvent fractions (Methanol, N- Hexane, Chloroform, And Aqeous) at concentrations ranging from 5 – 25 µg/mL. Results showed that methanol and aqueous extracts exhibited the highest antibacterial activity, with inhibition zones comparable to or greater than some conventional antibiotics (Amoxicillin, Tetracycline, Ciprofloxacin, Ceftriaxone and Ceftazidime). Antimicrobial susceptibility testing was performed using agar well diffusion methods. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were also determined. Phytochemical analysis revealed a higher concentration of phenols and glycosides in both plants, with methanol and water extracts showing the highest total bioactive content. Methanol extracts of *M. oleifera* exhibited the highest antimicrobial activity (overall mean: 11.92 ± 0.36 cfu/mL), compared to *O. gratissimum* (15.62 ± 0.59 cfu/mL). Additionally, n-hexane and chloroform extracts of *M. oleifera* demonstrated superior potency with mean inhibition values of 7.00 ± 0.32 cfu/mL and 10.85 ± 0.42 cfu/mL, respectively. In contrast, water extracts of *O. gratissimum* showed the highest activity (17.27 ± 0.80 cfu/mL), outperforming *M. oleifera* (14.56 ± 0.70 cfu/mL). MIC and MBC values supported these results. The overall susceptibility of bacterial isolates to plant extracts was 77.5 %, surpassing the 64.0 % susceptibility recorded for standard antibiotics. These suggest that *O. gratissimum* and *M. oleifera* may possess bioactive compounds with promising antibacterial properties.

Keywords: *Moringa oleifera*, *Ocimum gratissimum,* phytochemical, antibacterial effect, enteric bacteria

Word Count: 293

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List of Acronyms

Abbreviation	Meaning
E. coli	*Escherichia Coli*
CNS	Central Nervous System
GC-MS	Gas Chromatography-mass Spectrometry,
FTIR	Fourier-transform Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
ACE	Angiotensin-1-converting Enzymes
HIV	Human Immunodeficiency Virus
COX-2	Cyclooxygenase-2
TNFa	Tumor Necrosis Factor Alpha
IL-1b	Interleukin-1 beta
IL-6	Interleukin-6
Inos	Inducible Nitric oxide Synthase
NO	Nitric oxide
GK	Goto-Kakizaki
HGF	Hepatocyte Growth Factor
INH	Isoniazid
RMP	Rifampicin
PZA	Pyrazinamide
EBV	Epstein-Barr Virus

Chapter One
Introduction

1.1 Background to the Study

The administration of synthetic and chemically formulated drugs for treating bacterial infections often results in long-term complications for recipients. Many synthetic drugs exhibit severe side effects that can sometimes outweigh their benefits due to certain chemical components that may be carcinogenic, cytolytic, or cytotoxic when taken in high doses[1]. Consequently, the utilization of ethno-medicinal or natural plant-based alternatives is crucial to mitigate the adverse effects and toxicity associated with conventional drugs by adopting a natural approach to treatment[2].

Research on medicinal plants, herbs, and spices has been an integral part of human history for thousands of years and remains a significant subject of interest among life scientists today, particularly due to issues such as drug resistance and high pharmaceutical costs. Scientists, particularly in Africa and other developing regions, are actively investigating local plants used in traditional medicine[3]. Many synthetic drugs induce serious adverse effects in recipients, sometimes leading to temporary or permanent disabilities. Additionally, infections such as *gastrointestinal disorders, dysentery, diarrhea,* and *candidiasis* contribute significantly to morbidity and mortality rates, especially in tropical countries like Nigeria[4]. These ailments affect people at various stages of life, causing discomfort and distress and, in severe cases, necessitating hospitalization. The majority of pathogens responsible for gastroenteritis have developed resistance to numerous conventional drugs, making complementary and alternative therapies a promising avenue for future pharmacological advancements[5].

Throughout history, disease treatment has involved administering remedies derived from plants, animals, and minerals. The use of plant extracts for treating human ailments is an ancient practice[6]. The study of African medicinal plants for antimicrobial properties is among the most frequently conducted biological tests on various plant extracts. Compared to synthetic drugs, herbal medicines are often perceived as primitive and unscientific; however, they remain the primary healthcare option for approximately 75-80% of the world's population, particularly in developing nations[7].

Ocimum gratissimum, commonly known as scent leaf, is a perennial plant found abundantly in tropical regions of Africa and Asia. Belonging to the Labiatae family, it is the most widely available species of the *Ocimum* genus. In Nigeria, it is known as "effinrin-nia" by the Yoruba, "Nchonwu" in Igbo, and "Daidoya" by the Hausa. The plant can grow between one to three meters in height, with dark brown stems bearing leaves throughout. These leaves, typically green, are narrow and oval-shaped, measuring approximately 5-13 cm in length and 3-9 cm in width. The flowers are pale yellow, and the plant emits a distinctive camphor-like fragrance[8].

Traditionally, *Ocimum gratissimum* is widely used across West Africa as an antipyretic, antimalarial, and anticonvulsant. The crushed leaf juice is employed in managing convulsions, stomach aches, and catarrh, while the leaf oil has demonstrated antiseptic, antibacterial, and antifungal properties. In coastal regions of Nigeria, the plant is utilized to treat epilepsy, fever, and diarrhea, whereas in savannah areas, leaf decoctions are used for mental health treatments. Among the Igbo of southern Nigeria, the plant is commonly applied in neonatal umbilical cord care, believed to maintain sterility[9]. It is also employed for treating fungal infections, fever, cold, and catarrh. Clinical trials on dermatological creams formulated with *Ocimum gratissimum* extracts have yielded favorable outcomes, and tonics derived from the plant are used for treating skin infections, bronchitis, and conjunctivitis. Additionally, antiseptic preparations from this herb are effective in wound dressing, while its extracts serve as cough remedies[10].

Tea prepared from *Ocimum gratissimum* leaves is used for treating fever and inducing perspiration. The plant's volatile oils have significant antimicrobial properties, and its culinary importance is evident in its use as a spice for flavoring meat products[11]. Scent leaf decoctions are employed in treating *diarrhea, gonorrhea*, and *vaginitis* and are also used in mental health therapy[12]. Studies have also indicated that extracts from the plant help lower blood pressure, serve as potent insect repellents, and eliminate disease-causing microorganisms, including *Candida species*[13]. The plant's essential oil is particularly rich in eugenol, a phenylpropene compound responsible for many of its medicinal properties[15]. Research further confirms its effectiveness against bacterial pathogens such as *Shigella, Salmonella, Escherichia coli*, and *Proteus species*, as well as *E. coli* strains responsible for dysentery and typhoid[16]. *Ocimum gratissimum* extracts have also demonstrated effectiveness in veterinary medicine, including worm control in goats and libido enhancement in laboratory mice[17]. The plant's bioactive components contribute to its ability to relax intestinal muscles, reduce blood glucose levels, and prevent convulsions and seizures[18,19,20]. The adoption of natural treatments for infectious diseases is expected to shape the future of pharmacology by promoting the development of safer, low-toxicity therapeutic agents[21].

Moringa oleifera, commonly referred to as the "miracle tree," belongs to the *Moringaceae* family and is native to the Indian subcontinent[22]. It is often called the "drumstick tree," "horseradish tree," or "ben oil tree" due to its multiple applications[23]. Recognized for its immense medicinal and nutritional value, *Moringa* has been traditionally used to manage wounds, pain, ulcers, liver disease, heart disease, cancer, and inflammation. It thrives in various tropical and subtropical regions and is well-known for its ability to grow in arid and nutrient-deficient soils, earning it the nickname "never die[24]."

Moringa oleifera is a highly valued plant due to its nutritional and pharmacological properties. It is widely cultivated in tropical and subtropical regions worldwide, with origins traced to Afghanistan, Bangladesh, India, and Pakistan[25]. The *Moringa* genus includes 13 species, with *M. oleifera* being the most extensively used for its nutritional, medicinal, and agricultural benefits. Nearly every part of the tree is utilized, particularly for its essential nutrients[26]. *M. oleifera* leaves are rich in beta-carotene, minerals, calcium, and potassium. Dried leaves contain approximately 70% oleic acid, making them suitable for cosmetic applications[27]. The tree's bark is used in treating ulcers, toothaches, and hypertension, while its roots have applications in helminthiasis and paralysis treatments[28]. Moringa flowers are employed for treating ulcers, enlarged spleens, and aphrodisiac formulations. Due to its rich nutrient composition, the plant plays a vital role in alleviating malnutrition, particularly in infants and lactating mothers[29].

Moringa oleifera is highly regarded for its nutritional composition, attributed to its abundance of essential phytochemicals present in its leaves, pods, and seeds. It provides significantly higher amounts of key nutrients compared to common foods: seven times more vitamin C than oranges, ten times more vitamin A than carrots, seventeen times more calcium than milk, nine times more protein than yogurt, fifteen times more potassium than bananas, and twenty-five times more iron than spinach[30]. The leaves are rich in minerals such as calcium, potassium, zinc, magnesium, iron, and copper, as well as vitamins including beta-carotene (vitamin A), B-complex vitamins (such as folic acid, pyridoxine, and nicotinic acid), and vitamins C, D, and E. Moringa also contains beneficial phytochemicals such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids, and reducing sugars, along with anti-cancer agents like glucosinolates, isothiocyanates, glycoside compounds, and glycerol-1-9-octadecanoate. Its leaves contain zinc levels that meet daily dietary requirements[31].

Various parts of *Moringa oleifera* exhibit numerous medicinal properties, including cardiac and circulatory stimulation, anti-tumor, antipyretic, antiepileptic, anti-inflammatory, anti-ulcer, antispasmodic, diuretic, antihypertensive, cholesterol-lowering, antioxidant, anti-diabetic, hepatoprotective, antibacterial, and antifungal activities[32]. Aqueous extracts from the roots and bark prevent implantation, fruit extracts exhibit anti-inflammatory effects, methanolic leaf extracts display anti-ulcer activity, and ethanolic seed extracts possess anti-tumor properties[33]. The plant's bioactive compound, β-sitosterol, plays a crucial role in cholesterol stabilization. Additionally, its leaves, rich in β-carotene and lutein, prevent night blindness and other eye disorders. The plant juice is also effective in treating conjunctivitis. *Moringa oleifera* holds promise as a natural, reliable, and safe anti-cancer agent at appropriate concentrations[34].

1.2 Statement of the Problem

The global burden of antimicrobial resistance poses a significant threat to public health. Enteric bacteria, responsible for numerous gastrointestinal infections, have developed resistance to many conventional antibiotics. This resistance leads to treatment failures, prolonged illnesses, and increased healthcare costs.

While synthetic antibiotics remain the cornerstone of treatment, they are often associated with adverse effects and the rapid emergence of resistant strains. Ethnomedicinal plants like *Ocimum gratissimum* and *Moringa oleifera* have shown potential as sources of natural antimicrobials, but their comparative effectiveness against enteric bacteria is not well-documented. This study aims to compare the phytochemical and antibacterial properties of these plants to identify their promising alternative treatments.

1.3 Justification of the Study

The increasing prevalence of antibiotic resistance among enteric bacteria necessitates the search for alternative antimicrobial agents. *O. gratissimum* and *M. oleifera* are readily available, affordable, and widely used in traditional medicine, making them suitable candidates for this purpose. Other studies have reported their antimicrobial activity, but there are no reports on the comparison. This study will provide scientific validation for their traditional uses and offer insights into their relative efficacy, aiding in the prioritization of plant-based resources for further pharmacological development. The findings could contribute to the development of natural antimicrobial agents to combat resistant bacterial strains, thereby reducing reliance on synthetic antibiotics.

1.4 Aim and Objectives of the Study

The aim of the study is to evaluate the *in-vitro* phytochemical composition and antibacterial activities of *Ocimum gratissimum* and *Moringa oleifera* leaves against some selected enteric bacteria. To also report comparison between the two extracts.

The objectives of the study were to:

i. Identify the phytochemical constituents in the leaves of *Ocimum gratissimum* and *Moringa oleifera* using Gas Chromatography- Mass Spectroscopy.(GCMS)

ii. Assess the antibacterial activity of the extracts of these plants against selected enteric bacteria using the Kirby Bauer method.

iii. Compare the phytochemical and antibacterial profiles of the two plant extracts, being that work had not been done on comparison of both plants.

1.5 Research Questions:

1 What are the phytochemical constituents present in the leaves of *Ocimum gratissimum* and *Moringa oleifera*?

2 How effective are the extracts of *Ocimum gratissimum* and *Moringa oleifera* in inhibiting the growth of selected enteric bacteria?

3 Which plant, *Ocimum gratissimum* or *Moringa oleifera*, has superior antibacterial and phytochemical profiles?

1.6 Significance of the Study

This study will provide scientific validation for the traditional use of *Ocimum gratissimum* and *Moringa oleifera* in the treatment of bacterial infections and contributes to the growing body of knowledge on plant-based antimicrobial agents.

This study will offer potential solutions to the problem of antibiotic resistance by identifying natural and effective alternatives and the findings may inspire further research and development of plant-based pharmaceuticals.

1.7 Scope of the Study

The study focuses on the leaves of *Ocimum gratissimum* and *Moringa oleifera* and their *in-vitro* antibacterial activity against selected enteric bacteria, such as *Escherichia coli*, *Klebsiella spp.*, *Staphylococcus aureus*, *Salmonella spp.*, and *Pseudomonas aeruginosa*. The study also involves the phytochemical analysis of the plant extracts to identify bioactive compounds responsible for their antibacterial properties.

1.8 Limitations of the Study

This study is limited to the leaves of *Ocimum gratissimum* and *Moringa oleifera*, excluding other parts of the plants that may also exhibit antimicrobial properties. The antibacterial activity is assessed *in-vitro*, which may not usually represent the *in-vivo* effects of the plant extracts.

1. Only a limited number of enteric bacteria are tested, which may not capture the full spectrum of the plants' antibacterial potential.
2. The study relies on laboratory conditions and equipment, which may not replicate natural or clinical environments.

1.9 Operational Definition of Terms

Phytochemical Constituents: Bioactive compounds found in plants, such as alkaloids, flavonoids, tannins, and saponins, which contribute to their medicinal properties.

Antibacterial Activity: The ability of a substance to inhibit the growth or kill bacteria.

Enteric Bacteria: Bacteria that reside in the intestinal tract, some of which can cause gastrointestinal infections.

In-vitro: Laboratory-based experiments conducted outside a living organism, typically in controlled conditions such as test tubes or petri dishes.

Ocimum gratissimum: A medicinal plant commonly known as scent leaves, widely used for its therapeutic properties.

Moringa oleifera: A fast-growing tree known as *moringa*, with numerous medicinal and nutritional benefits.

Comparative Evaluation: The process of analyzing and comparing two or more variables to determine their relative effectiveness or properties.

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Chapter Two

Literature Review

Pathogenic gastrointestinal microorganisms are those that cause gastroenteritis[1]. They infect the gut, causing inflammation of the stomach and intestine. This cause vomiting, severe abdominal pains, and diarrhea. They consist of *Escherichia coli, Shigella species*, and *Salmonella species*. Bacterial gastroenteritis is usually caused by improper hygiene practices[2]. Infections can also occur as a result of direct contact with infected animals or from consuming food or water contaminated with bacteria or poisonous compounds produced by bacteria. The emergence of antibiotic resistance, as well as the recent unfavorable side effects of some commercially available antibiotics, has prompted the screening of plant extracts in order to discover new drugs that could serve as alternative therapies for the treatment of various infections and diseases[3].

Antibacterial are antimicrobial agents that are used to treat bacterial infections. Antibiotics (a chemical produced by microbes that inhibits or kills another germ at a very low concentration) had been useful in the control of pathogenic bacteria until recently, when most pathogens developed resistance[4]. Antibiotic resistance is becoming a global issue, posing a threat to humanity, as many diseases and infections that were previously treated with this chemical are now difficult to control[5]. Furthermore, several synthetic antibacterial medicines are losing efficacy due to side effects include tendinitis, seizures, and Steven-Johnson syndrome.

The growth of antibiotic resistance, as well as the recent unwanted side effects of some commercially available antibiotics, has prompted the screening of plant extracts in search of new drugs that could serve as alternative therapy for the treatment of a variety of illnesses and diseases[6].

African traditional medicine is the oldest medicinal system and is commonly referred to as the

Cradle of Mankind[7]. For thousands of years, traditional herbal treatments have been utilized to treat infectious disorders all throughout the world[8]. Because mainstream medicine is not widely used, there has been a revived interest in traditional medicine around the world[9]. In disadvantaged countries, health care has been supported by culturally appropriate approaches. In many developing countries, including Nigeria, 80% of patients utilize traditional herbal medicines to manage infectious diseases[10]. Despite the availability of modern treatment in some cultures, herbal medicines (medicinal plants) remain popular for historical and cultural reasons, as well as their efficacy and lower cost[11]. They also represent potential sources of new medicinal chemicals, as all portions of a plant, from roots to seed heads and flowers, are used in traditional treatments and hence can operate as sources of lead compounds[12]. Furthermore, compounds derived from natural products have accounted for around 80% of medications introduced to the market[13].

The global interest in using plant-based remedies has been on the rise in recent years, alongside the exploration for new phytochemicals that could be developed into effective treatments for infectious diseases[14]. Despite the extensive ethnobotanical documentation, there is a lack of scientific data regarding the efficacy and phytochemistry of indigenous medicinal plants in Nigeria[15]. Historical records indicate that the first studies on the antimicrobial properties of Nigerian plants were conducted by Dalziel in 1937 and 1957. It was not until approximately twenty years later that a handful of additional publications on the chemistry and antimicrobial effects of Nigerian plants emerged in scientific literature[16]. During the 1980s, only a limited number of studies concerning the antimicrobial activity of Nigerian plants made their way into existing literature.

Since 1990, there has been a significant increase in publications focusing on the chemistry and antimicrobial properties of medicinal plants from Nigeria[17]. This rising interest in scientifically validating the antimicrobial effects of these plants may stem from heightened public awareness, advances in methodologies, and numerous citations in local literature highlighting the necessity for such research[18]. Additional motivations for the advancement of studies on Nigerian medicinal plants include the quest for new lead compounds to develop into drugs or serve as templates for synthesizing analogs, as well as the assessment of traditional medicine and herbal products[19]. The medicinal properties of Nigerian plants are believed to result from the interaction of various phytochemicals (including alkaloids, tannins, phenols, saponins, flavonoids, and essential oils) and bioactive compounds present in their tissues. Lately, the pharmaceutical and scientific communities have shown increased interest in medicinal plants, as evidence has highlighted the promising potential of antimicrobial substances derived from plants[20].

The antimicrobial properties of plant extracts have been foundational to many medical applications like pharmaceuticals, alternative medicine, and natural therapies since ancient times. In traditional medicine, the use of plant materials is widespread, particularly in developing nations, where they are the primary resources available for treating various infections and health issues in rural areas[21].

Plants possess a wealth of secondary metabolites and are a significant source of chemical diversity, making them potential candidates for new drug development[22]. Foodborne pathogens pose a considerable health challenge in developing countries, with *Salmonella, Staphylococcus aureus*, and *E. coli* being among the most prevalent pathogens responsible for food poisoning and related infections worldwide[23]. The prevalence of antimicrobial drug-resistant strains among these foodborne pathogens, particularly *E. coli, S. aureus,* and *Salmonella spp*, is increasing due to the misuse of antibiotics intended for human treatment applied in animal husbandry and other veterinary practices. Such practices create a selective pressure that allows resistant microbes to thrive and spread throughout communities[24]. Multidrug-resistant *Salmonella* are often isolated from food sources.

Humans have utilized medicinal plants for treating illnesses for thousands of years. Estimates suggest that more than two-thirds of plant species worldwide possess medicinal properties. The World Health Organization estimates that about 80% of individuals living in developing nations rely on traditional medicine for their fundamental healthcare needs. Approximately 50 to 60% of pharmaceutical products are derived from natural sources or synthesized from them. Nevertheless, many plants still lack a clear understanding of their mechanisms of action, biological targets, and possible side effects of their constituents[25]. Despite this knowledge gap, a significant portion of the population continues to use them for self-medication, which can lead to severe and sometimes permanent harm[26]. Therefore, it is imperative to conduct pharmacological research to evaluate the efficacy of these plants used in traditional medicine[27].

A medicinal plant is defined as any plant possessing one or more organs that contain substances suitable for therapeutic use or that can serve as precursors for synthesizing antimicrobial drugs[28]. Presently, plants serve as the primary medicinal resource for individuals of all ages across many countries, where various diseases are mainly treated with traditional remedies derived from plants. The modern pharmaceutical industry continues to heavily depend on the variety of secondary metabolites found in plants, of which at least 12,000 have been isolated, a figure estimated to represent less than 10% of the total diversity[29].

In plants, the synthesized aromatic substances (metabolites) are used as defensive weapons against predation by microorganisms, insects and herbivores[30]. However, some of these metabolites are involved in pigmentation (tannins and quinines) and formation of plant odour (terpenoids) and flavour (capsaicin). These defensive molecules give plants their medicinal value which is appreciated by human beings because of their importance in health care of individuals and communities. The search for plants with antimicrobial has gained increasing important in recent years due to the development of antimicrobial drug resistance and often the occurrence of undesirable side effect of some antibiotics [31].

With the recent advent of ever-increasing resistant bacteria, there has been a corresponding rise in the universal demand for natural antibacterial therapeutics[32]. Although pharmacological industries and researchers have produced a good number of antibiotics in the last three decades, resistance to these drugs by microorganisms is increasingly high. Several plants have therapeutic and pharmaceutical effects, for antimicrobial, antioxidant, anti-infectious and anti-tumour activities [33]. Herbal medicine has been widely used as an integral part of primary health care in many countries was reported. Medicinal plants may constitute a reservoir of new antimicrobial substances to be discovered[34].

About 80% of developing country's citizens use traditional medicine based on plant products. Thus, many studies have been conducted on medicinal plants[35]. They are screened for antimicrobial activities, their properties and efficacy. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activities. It also facilitates pharmacology studies[36]. Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, ageing process and perhaps dementias Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties. Many human diseases are caused or negatively affected by free radicals [37].

Plant-derived products are now gaining recognition worldwide currently recommended for primary healthcare, but only a few plants have received rigorous scientific investigation[37]. Medicinal plants are natural products used since time immemorial for the treatment of various human diseases. All over the world, the citizen and health practitioners are now starting to rely on herbal medicines as a substitute for scientifically proved therapies[38]. Herbal products contained secondary metabolites that are synthesized through the pentose phosphate, shikimic acid, and phenylpropanoid pathways. These compounds play a significant defensive role against pathogenic bacteria and oxidative stress caused by abiotic stress in plant [39]. Currently one of the strategies used to treat skin and oral infections is the use of natural products from plant origin perhaps due to the perception that long-term use of western medicine induces severe complications[40]. Consequently, many Nigerian patients, in particular, those living in the rural areas, have resorted to seeking relief via traditional healers and herbalists who administer herbal drugs in the management of human diseases[41].

In plants, aromatic compounds (metabolites) serve as protective agents against attacks from microorganisms, insects, and herbivores. Some of these compounds also contribute to the coloration (tannins and quinones) and the production of scents (terpenoids) and flavors (capsaicin) in plants[30]. These protective substances grant plants their medicinal properties, which are valued by humans due to their role in individual and community health care[31]. The quest for plants with antimicrobial properties has gained significant importance recently, particularly because of the rise in antimicrobial drug resistance and the frequent undesirable side effects associated with certain antibiotics[32].

As antibiotic resistance among bacteria continues to grow, there has been an increasing global demand for natural antibacterial treatments. While the pharmaceutical industry and researchers have developed a considerable number of antibiotics over the past thirty years, microorganisms are exhibiting ever-increasing resistance to these drugs[33]. Numerous plants show therapeutic and pharmaceutical benefits, offering antimicrobial, antioxidant, anti-infectious, and anti-tumor effects. Herbal medicine has long been a vital component of primary health care in numerous countries. Medicinal plants may serve as a source of new antimicrobial compounds yet to be discovered[34].

Approximately 80% of the population in developing nations relies on traditional medicine derived from plant sources[35]. Consequently, extensive research has been conducted on medicinal plants, screening them for their antimicrobial properties, effectiveness, and characteristics. Extracting bioactive compounds from these plants enables the demonstration of their physiological effects and enhances pharmacological studies[36]. Antioxidants act as radical scavengers, safeguarding the human body against free radicals that can lead to health issues like ischemia, anemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's disease, aging, and possibly forms of dementia. Flavonoids and flavones, widespread secondary metabolites, possess antioxidant and antiradical capabilities. A variety of human health problems are caused or worsened by free radicals[37].

Globally, plant-based products are increasingly acknowledged and currently recommended for primary healthcare, although only a limited number of these plants have undergone thorough scientific examination. Medicinal plants have been used for centuries as natural remedies for a range of human ailments[38]. Around the world, both citizens and health practitioners are increasingly turning to herbal remedies as alternatives to scientifically validated treatments. Herbal products contain secondary metabolites that are produced through the pentose phosphate, shikimic acid, and phenylpropanoid pathways[39]. These compounds play a crucial defensive role against harmful bacteria and the oxidative stress caused by abiotic stressors in plants. Presently, one approach to treating skin and oral infections involves utilizing natural products derived from plants, possibly due to the belief that prolonged use of conventional medicines can lead to serious complications[40]. As a result, many patients in Nigeria, especially those in rural regions, have begun to seek relief from traditional healers and herbalists who provide herbal medications for managing health conditions[41].

The practice of using plants to cure ailments dates back to the earliest days of humanity[42]. Observations regarding the application and effectiveness of medicinal plants significantly aid in uncovering their therapeutic properties, which results in their frequent prescription, even when their chemical components are not fully understood[43]. Research has demonstrated varying levels of antibacterial and antifungal activity against specific pathogens. Flavonoids have been discovered to show enhanced antifungal and antibacterial effects against various human pathogenic fungi and bacteria [44,45]. Medicinal plants host a diverse array of secondary metabolites or compounds, including tannins, terpenoids, alkaloids, and flavonoids, which significantly influence the plants' therapeutic effectiveness, especially regarding antimicrobial properties[46]. Comparable phytochemical constituents, such as flavonoids and tannins, have also been demonstrated to be effective against pathogenic bacteria like *Bacillus cereus* and *Staphylococcus aureus*[47]. The tannins contained in these medicinal plants make them valuable for producing antiseptic soaps commonly used for bathing or cleansing skin surfaces[48]. Literature indicates that phytochemicals may exhibit toxicity to filamentous fungi, yeasts, and bacteria while also inhibiting viral reverse transcriptase activity. Saponins have been identified as a key antifungal secondary metabolite[49]. A broad range of physiological actions of saponins, steroids, phenols, and tannins appear to dominate and could be responsible for the antimicrobial effects observed[50].

In West Africa, particularly Nigeria, medicinal plants display unique characteristics in the realm of herbal therapy[51]. There are around 1,000 medicinal plants found in Nigeria, yet the majority of their medicinal properties have yet to be explored[52]. The therapeutic potential of these plants might play a critical role in addressing current or future health issues[53]. Some medicinal plants can enhance, counteract, or neutralize potential adverse effects within the body and are classified as synergistic medicinal plants; others are utilized for the treatment of complex conditions, such as cancer, and are known as official medicinal plants; while some possess the ability to prevent the onset of certain diseases by mitigating the side effects of synthetic treatments, categorizing them as preventive herbal medicinal plants[54].

2.1 Role of Medicinal Plants in Traditional Healing in Nigeria

The introduction of plant derived drugs in modern medicine has been linked to the uses of plant derived materials as an indigenous cure in traditional system of medicine [55,56]. Some of the plants have been found to possess significant antibacterial, antifungal, anticancer, antidiuretic, anti-inflammatory and anti-diabetic properties [57,58,59]. Some other uses of herbal medicines are in venom neutralization by lupeol acetate isolated from the root extract of *Hemidesmus indicus*, treatment of hypertension and lowering of blood sugar by serpentine isolated from the root of *Rauwolfia serpentine*, treatment of Hodgkin's, choriocarcinoma, non-Hodgkin lymphomas, leukemia in children, testicular and neck cancer from vinblastine isolated from the *Catharanthus rosesus* [60,61], treatment of acute lymphocytic leukemia in childhood advanced stages of

Hodgkin's, lymophosarcoma, cervical and breast cancer amongst others. Plant derived drugs are used to cure mental illness, skin diseases, tuberculosis, diabetes, jaundice, hypertension and cancer[62].

Medicinal plant contains chemical compounds that dictate their therapeutic potency. Researchers have shown that different plants contain different bioactive components at different concentrations[63]. The higher the amount of the important phytochemical in medicinal plants, the greater therapeutic potency or medicinal importance of the plants[64]. There are more than 300 known medicinal plants in Nigeria; though the applications vary from plants to plants, culture and people believe, weather and other factors, many of these plants are not widely distributed; some grows well in rain forest while others in savannah regions[65].

2.2 Phytochemical Components of Medicinal Plants

Medicinal plants harbor bioactive organic chemical compounds commonly known as phytochemicals, which serve a protective function against significant chronic diseases, including both host-metabolic or genetic disorders and infectious diseases, and are found in grains, vegetables, fruits, and various other plant products[66]. Phytochemicals engage in metabolic processes as intermediates and act as primary metabolites like fats and sugars present in all plants, whereas secondary metabolites occur in a limited number of plants and serve specialized roles[67].

Secondary metabolites are compounds produced by plants to enhance their survival or adaptation within their environment, responding to numerous factors such as drought resistance, pollination, and protection from predators, although they are not essential for the immediate survival of the plants[68]. Due to their therapeutic properties in humans, secondary metabolites and pigments are converted into pharmaceuticals, including inulin (from dahlias), morphine and codeine (from the poppy), quinine (from cinchona), and digoxin (from foxglove)[69].

Analysis of the chemical makeup of medicinal plants has shown that they contain a variety of bioactive compounds, which feature saponins, tannins, and alkaloids[70]. The principal functional categories of phytochemicals with potential therapeutic benefits encompass antioxidants, anticancer substances, immunity-enhancing agents, detoxifying compounds, and neuroactive agents[71]. Plants generate distinctive combinations of phytochemicals that often fall within established biochemical frameworks[72]. Among these bioactive compounds, triterpenoids exhibit anti-inflammatory properties, while tannins possess astringent, anti-inflammatory, and antimicrobial effects[73]. Saponins are recognized for their medicinal properties, acting as blood purifiers, expectorants, and antibiotics. Meanwhile, alkaloids significantly impact the central nervous system (CNS), and glycosides are noted for their ability to enhance systolic contraction strength[74].

2.3 Phytochemical Screening of the Medicinal Plants

The bioactive compounds found in medicinal and aromatic plant extracts are typically present in different plant parts, including roots, stems, bark, leaves, and flowers[75]. Often, the effects of these compounds on the human central nervous system (CNS) can be linked to their ecological roles or biochemical functions in other plant species and higher organisms[76]. Extracting and utilizing these biologically active substances from plant sources involves several key steps, such as extraction, preliminary screening, isolation, purification, characterization, and both pharmacological and toxicological assessments[77]. In pharmaceutical applications, extraction refers to the separation of bioactive components from plant or microbial tissues using selective buffer solutions or solvents, following established protocols[78]. Various factors influence the quality of plant extracts, including the specific plant part used (such as leaves, bark, or roots), the type and concentration of the solvent, and the extraction method[79]. Additionally, the chemical profile of the extract is affected by the nature of the plant material, its source, the degree of processing, moisture content, and particle size. Other parameters that determine the composition and quantity of secondary metabolites in an extract include extraction method and duration, solvent type and concentration, processing temperature, and analyte polarity[80].

Phytochemical screening of medicinal plants is commonly conducted against a broad spectrum of microorganisms to evaluate their antimicrobial potential, which is largely attributed to their secondary metabolites[81]. The growing issue of antimicrobial resistance among bacteria has heightened the urgency for ongoing research into safer and more effective therapeutic agents.

Many plants with traditional medicinal uses have been documented for their promising activity against various disease-causing microorganisms[82]. Research continues to advance the functional understanding of medicinal plants, contributing to the development of approximately 25–50% of commercially available drugs[83]. The antimicrobial properties of these plants vary; some work synergistically to enhance therapeutic efficacy and reduce the adverse effects of synthetic drugs, while others function as quorum quenchers[84].

A range of analytical techniques is employed to purify, identify, and characterize the structural components of phytochemicals in plant extracts[85]. These techniques include different types of column chromatography, gas chromatography-mass spectrometry (GC-MS), Fourier-transform infrared spectroscopy (FTIR), ultraviolet (UV) spectrophotometry, and nuclear magnetic resonance (NMR) spectroscopy, which are used sequentially for precise analysis[86].

2.4 Effect of Processing on Phytochemicals

The way medicinal plants are processed can affect their antioxidant activity by either increasing or decreasing their effectiveness[87]. Different processing techniques for extracts of sandpaper leaf (Ficus exasperata) demonstrated inhibition of arginase and angiotensin-1-converting enzyme (ACE) activities in a dose-dependent manner. Furthermore, their findings indicated that soaking resulted in a significantly greater inhibition of both enzymes compared to methods such as hand maceration, boiling, and blending[88]. Previous research indicates that the health benefits of medicinal plants, whether in single or mixed formulations, often arise from the complex combinations of phytochemicals that work together to target multiple pathways, resulting in collective therapeutic effects due to their combined antioxidant capacities[89]. The impact of various processing methods, including blanching, cooking (thermal processing), dehydration, extrusion, and irradiation, alongside nonthermal processing methods (such as storage and carbon dioxide treatment), as well as other formulation ingredients on phytochemicals, has been thoroughly studied[90]. However, it is important to note that during processing, naturally occurring antioxidants can degrade through mechanisms like Strecker degradation and Maillard reactions, leading to the formation of new compounds with either reduced or enhanced antioxidant activity[91]. This accounts for the variations in total phenolic content and antioxidant activities observed in processed foods when compared to their unprocessed counterparts[92].

2.5 Mechanisms of Action of Medicinal Plants

The infrequent instances of infectious diseases in wild plants suggest the existence of effective defense mechanisms. Phytochemicals can exert antimicrobial effects through several mechanisms, including (i) inhibition of enzymes and toxins, (ii) damage to bacterial membranes, (iii) suppression of virulence factors, (iv) biofilm formation, (v) inhibition of protein synthesis, and (vi) quorum quenching. Tannins primarily function through their capacity to bind with proteins, thereby preventing protein synthesis within the cells[93]. The interference with quorum sensing occurs at three levels: (i) signal synthesis, (ii) signal sequestration, and (iii) signal reception[94].

While antibiotics have historically been effective against infectious diseases, the development of resistance to these drugs has led to the emergence of new infections and the return of previously controlled diseases[95]. Bacterial resistance to antibiotics can be attributed to three main factors: (i) the direct destruction or modification of the antibiotic by enzymes produced by the bacteria, (ii) alterations to the target that reduce the drug's binding efficiency, and (iii) the efflux of the antibiotic from the bacterial cell[96].

One strategy to combat resistance mechanisms in combination therapies involves using combined active drugs or polyherbal formulations[97]. The incorporation of natural products alongside antibiotics to enhance treatment effectiveness represents a novel approach developed to tackle antibiotic resistance[98]. The multitude of bioactive compounds found in medicinal plants offers a synergistic therapeutic effect, and when used concurrently with standard medications, these plants can often amplify the drug's effectiveness while addressing concerns related to toxicity and overdose[99].

2.6 *Ocimum gratissimum* (Scent Leaves)

From ancient times, people have utilized medicinal plants within traditional and complementary medicine to heal, manage, or avert various health conditions. It is estimated that 80% of the global population relies on ethnomedicine or herbal remedies, primarily to address numerous ailments worldwide. *O. gratissimum*, commonly known as scent leaf due to its fragrance, is frequently employed as a spice in food and soup preparation in Nigeria. This medicinal herb has been traditionally utilized for treating various infections[100]. The plant is abundantly cultivated in different regions of Nigeria and contains several bioactive compounds, such as tannins, saponins, alkaloids, glycosides, phenols, and flavonoids, collectively known as phytochemicals[101].

Scent leaf, scientifically referred to as "Ocimum gratissimum," is a plant that is widely grown for both culinary and medicinal purposes in West Africa, typically found in and around village huts and gardens. The leaves, which possess a strong aroma, are commonly used to season meat, especially game, and to enhance the flavor of soup[102]. They serve as an essential ingredient in soups like "pepper soup" and various similar dishes across southeastern Nigeria and beyond. Furthermore, over the last decade, many researchers have shown interest in medicinal plants and their bioactive components due to their potential in managing and preventing life-threatening and chronic diseases such as arthritis, diabetes, cancer, and stroke[103]. It is a perennial herbaceous plant characterized by a strong scent that also has commercial viability[104]. Scent leaf grows in Africa, Asia, and South America, and belongs to the Lamiaceae family. Additionally, scent leaf is used to enhance the flavor of fish, meat, soups, and stews, and it is also utilized in treating various conditions, including aches, fever, inflammation, anemia, diarrhea, as well as fungal and bacterial infections[105]. The leaves are packed with a range of macronutrients that are beneficial to the body. When consumed in moderation and appropriate amounts, the leaves offer significant nutritional value[106].

2.7 Traditional uses of Scent Leaf

The basil species known as *O. gratissimum* is often called clove basil, African basil, and wild basil[107]. It originates from southern Asia, Africa, Madagascar. Naturalized locations include Polynesia, Hawaii, Mexico, Panama, the West Indies, Brazil, and Bolivia[108]. The leaves of *O. gratissimum* (scent leaf) are primarily used in culinary applications. As a spice, *Ocimum* *gratissimum* is classified among plants known for their aromatic properties. This plant typically grows upright and has numerous branches, generally reaching heights of no more than one meter. It belongs to the Labiatea family and is categorized as a gratissimum species. While it is commonly found in the tropics and subtropics, it is particularly diverse in tropical Africa and India. Although commercially cultivated in Vietnam, it is often grown as a home garden crop throughout Southeast Asia. The plant serves various purposes; for example, different parts are utilized in treating conditions such as headaches, fevers, and asthma[109].

In many parts of the globe, scent leaves are incorporated into salads, soups, pastas, vinegars, and jellies. The Thai people, in particular, are familiar with using it as a seasoning in their dishes. In addition to its frequent applications in contemporary medicine, extracts from scent leaves have traditional uses in addressing ailments like fevers, coughs, and body pain[110]. The essential oil derived from *O. gratissimum* leaves contains significant concentrations of eugenol, thymol, camphor, pinene, limonene, and other chemical compounds, which contribute to its numerous medicinal benefits[111].

2.8 Health Benefits of Scent Leaf

Research has demonstrated that consuming the stems of *O. gratissimum* leaves can help in preventing bad breath and maintaining strong teeth due to the presence of oral microorganisms that combat tooth decay. Scent leaves offer a wide array of health benefits[110]. They contain calcium and magnesium, which help to reduce blood sugar levels. Furthermore, they promote heart health by eliminating unnecessary toxic substances and cholesterol from the body[111].

O. gratissimum aids in bowel movements, providing a soothing effect on the stomach while impproving the digestion process[112,113]. Recent studies indicate that *O. gratissimum* is used as a treatment for individuals living with AIDS and Human Immunodeficiency Virus (HIV)[114]. Scent leaves can be effective in alleviating stomach pains, dysentery, diarrhea, and vomiting[115]. They also facilitate quick digestion of food. Regular consumption of scent leaves can normalize blood sugar levels, showcasing one of their remarkable health benefits. Additionally, scent leaves can help repair the damage caused by nicotine and smoking in the body[116].

Ocimum gratissimum offers a variety of therapeutic benefits that have been used in traditional medicine[117]. While many perceive diarrhea as a slight, temporary issue, for others, it poses a significant and persistent problem. In fact, the World Health Organization identifies diarrhea diseases as the second leading cause of death among children under the age of five[118]. The use of scent leaf is among many treatments for diarrhea and recent studies indicate it can be effective in alleviating this condition. The active ingredient in *Ocimum gratissimum* is eugenol, which possesses antibacterial and antispasmodic properties. This means it can help eliminate the bacteria causing diarrhea while also relieving the cramps and bloating that often accompany it.

Traditionally, scent leaf (*O. gratissimum*) extract has been employed to treat ulcers due to its anti-inflammatory, analgesic, and anti-ulcer benefits[119]. The specific compounds believed to contribute to the anti-ulcer effects of scent leaf extract are eugenol and carvacrol, both of which are phenolic compounds[120]. Eugenol is a natural compound known for its anti-inflammatory and antioxidant effects, and it also inhibits the activity of cyclooxygenase-2 (COX-2), a key enzyme involved in ulcer formation. Carvacrol, another phenolic compound in scent leaf extract, is recognized for its antimicrobial, antioxidant, and anti-inflammatory properties[121].

It is thought to safeguard the gastric mucosa by enhancing mucus production and lowering gastric acid secretion. These compounds work together synergistically to shield the gastric mucosa from harm and promote the healing of existing ulcers[122]. Scent leaf is also referred to as the “fever” leaf due to its oil's antiseptic, antifungal, and antibacterial properties[123].

Figure 2.1: *Ocimum gratissimum* (Scent Leaves) [116]

2.9 Phytochemical Constituents of *Ocimum gratissimum*

A variety of phytochemical compounds can be found in scent leaves, each with specific concentrations despite belonging to different classes, and they offer numerous benefits[124].

2.9.1 Flavonoid

Flavonoids, which represent the largest group of naturally occurring phenolic substances, can be located in various parts of plants in both free forms and as glycosides. These metabolites, which have evolved from flavone, are known as flavonoids and are recognized for their antioxidant properties[125]. Generally, they feature a benzopyrone structure with phenolic or polyphenolic groups in different positions[126]. *Ocimum gratissimum* (scent leaves) is rich in phenolic compounds like rosmarinic acid, sinapic acid, salvigenin, garlic acid, catechins, methyl eugenol, caffeic acid, l-caftaric acid, trans-ferulic acid, lchicoric acid, and flavonoids such as xanthomicrol, cirsimaritin, rutin, apigenin, kaempferol, and vixen[127].

Due to wide usage in the food, feed, pharmaceutical, and medical sectors, there has been significant interest in the identification and analysis of polyphenols. The diverse subclasses of flavonoids include anthoxanthins (flavanone and flavanol), flavanones, flavanonols, flavans, chalcones, anthocyanidins, and isoflavonoids[128]. Flavonoids are plentiful in nature, predominantly found in various plants, fruits, herbs, stems, cereals, nuts, vegetables, flowers, seeds, and leaves, and possess promising applications in medical chemistry[129].

Flavonoids and phenolic compounds exert their anti-inflammatory and antioxidant effects through several mechanisms, such as scavenging or neutralizing free radicals, chelating metal ions, or inhibiting enzyme systems responsible for free radical production[130]. Recent studies on flavonoids have focused on their isolation, the synthesis of analogs, and the exploration of their effects on human health through various techniques and animal models. In experimental animal studies, certain plant-derived dietary flavonoids have been shown to hinder tumor growth[131]. Various biological activities of flavonoids have been discovered, including the inhibition of protein kinase C, mitochondrial adhesion reduction, antimicrobial activity, ulcer prevention, alleviation of arthritic pain, and inhibition of angiogenesis[132]. Moreover, these compounds contribute to cardioprotection and neuroprotection while also enhancing cognitive performance during the aging process. Consuming more flavonoid-rich foods offers numerous health benefits[133]. There has been an increased effort to extract these natural compounds from different plants due to their positive effects on human health. For example, scent leaves contain a high concentration of flavonoids, including Nepetrin, Quercetin, Rutin, and Catechin. Quercetin, a naturally occurring flavonoid found in plants (scent leaves), has shown efficacy in treating colorectal cancer[134]. The chemo-preventive actions of quercetin in colorectal cancer involve several mechanisms, such as cell cycle arrest, increased apoptosis, antioxidant effects, modulation of estrogen receptors, regulation of signaling pathways, and inhibition of metastasis and angiogenesis. Furthermore, quercetin aids in lung cancer reduction[135].

Numerous studies have indicated that the anticancer properties of flavonoids result from their ability to inhibit protein kinases, which are essential in regulating cellular pathways. The flavonoid quercetin is recognized as an effective anticancer agent and hinders the function of protein disulfide isomerase (PDI)[136]. Spectroscopic methods were utilized to analyze the composition of the flavonoids extracted from the leaves of the plant. The purified compound showed enzyme inhibitory activity and holds potential in cancer treatment. When quercetin, an antioxidant flavonoid, is present in the circulation from plants like *Ocimum gratissimum*, it supports vascular health and reduces the risk of cardiovascular illnesses[137]. It also helps prevent blood clotting and the likelihood of stroke, thanks to the presence of quercetin in *Ocimum* *gratissimum* and its derivatives. Rutin, a type of flavonol found in scent leaves, has demonstrated various biological effects, including cytoprotection, antioxidation, and anticancer activity. Incorporating these scent leaves and other aromatic herbs into daily meals will enhance the nutritional value of food while contributing additional flavor and aroma[138].

Research and clinical trials have shown that a blend of flavonoids can enhance venous function, minimize capillary permeability, and promote lymphatic and hemorrhoidal drainage[139]. Therefore, flavonoids are beneficial in managing hemorrhoids and bleeding disorders. It has been extensively documented that the natural bioactive flavonoids derived from medicinal plants and herbs, such as *Ocimum gratissimum* (scent leaves) and others, possess antiviral properties. These flavonoids have been concentrated and modified for improved efficacy[140].

2.9.2 Glucosinolate

Glucosinolate refers to any glucosinolate where the sugar component is glucose. It is widely accepted that glucosinolate contributes to the unique scent and flavor attributes of Brassicaceae crops[141]. The sulfur and nitrogen-containing glycosides known as glucosinolates are responsible for the distinctive taste of Brassicaceae vegetables (like horseradish, nasturtium, cabbage, broccoli, Brussels sprouts, and mustard), making them popular in human diets. The glucosinolate itself does not have a strong flavor[142]. The enzyme myrosinase, typically located in a different part of the cell, interacts with it, leading to the production of an aglycone, which generally undergoes rearrangement to form a harmful and destructive isothiocyanate[143]. While the aglycones of glucosinolates are water-soluble, agylcone are hydrophobic. These aglycones often referred to as mustard oils, possess a viscous oil-like consistency. Isothiocyanates are also known as mustard oil when they are created from glucosinolates via steam distillation.

Glucosinolates are typically not active at a physiological level, but when plant tissues are damaged, they undergo hydrolysis, resulting in a variety of structurally diverse hydrolysis products that possess different biological activities [144,145,146]. Studies indicate that, prior to forming a glucosinolate, a protein amino acid can experience several elongation processes[147]. Following this, the glucosinolate may undergo various metabolic changes, including hydroxylations, methylations, and oxidations. Additional research suggests that glucosinolates offer benefits such as antioxidant effects, inherent antibacterial properties, and roles in regulating inflammation, stress response, metabolism, and antioxidant functions[148]. It has also been shown that livestock consuming high-glucosinolates diets may encounter adverse effects, including reduced feed intake and growth, gastrointestinal discomfort, goiter, anemia, as well as liver and kidney lesions[149]. Therefore, consuming excessive amounts of Brassicaceae crops is not advisable, despite their significance [150].

Glucosinolates represent a small but chemically diverse category of protective secondary metabolites present in plants[151]. Moreover, glucosinolates serve as effective biopesticides, playing a role in a dual chemical defense system that protects plants against environmental stressors such as insect herbivory and disease. Certain studies reveal that plants that produce glucosinolates hold considerable economic and nutritional value, demonstrating resilience across various industrial, agricultural, and phytopharmacological applications. Research has also identified that the breakdown products of glucosinolates act as potent chemoprotective agents in humans, helping to combat the development of several chronic diseases, including cancer, cardiovascular conditions, and neurological disorders[152].

Excessive intake of glucosinolates may cause nausea and, in severe cases, vomiting. In the absence of medical assistance, this characteristic is sometimes exploited to induce vomiting by administering large quantities of mustard powder. In undamaged plants, thio glucosinolate, commonly referred to as myrosinase, and glucosinolate are physically separated, residing in the cytoplasm and vacuoles, respectively. When plant cells are damaged, myrosinase hydrolyzes glucosinolate, leading to the formation of appropriate nitriles, thiocyanates, and isothiocyanates, along with glucose and sulfate by-products[153]. The hydrolysis products of glucosinolate are well-known for contributing to the plant's distinct scent and flavor characteristics. Byproducts of glucose hydrolysis also possess antifungal, antibiotic, and insecticidal properties, which enhance the plant's overall defense mechanism[154].

2.9.3 Terpenoid

In a study conducted, various terpenes commonly found in forests were categorized based on their anti-inflammatory, anti-tumorigenic, or neuroprotective properties[155]. Additionally, the research delves into potential mechanisms through which terpenes operate and their effects on processes observed in different in vivo and in vitro systems. The findings that highlight the biological efficacy of terpenes support the health benefits associated with forest bathing and propose the potential of utilizing terpenes as chemotherapeutic agents for various human diseases[156].

Various plants, such as *O. gratissimum*, produce terpenes. Recent findings indicate that terpenes possess anti-inflammatory properties by inhibiting various pro-inflammatory pathways in conditions like osteoarthritis, bronchitis, ear edema, and chronic obstructive pulmonary disease[107]. Tumorigenesis is a multifaceted process, characterized by several traits such as uncontrolled cell proliferation, irregular apoptosis, invasion activation, angiogenesis induction, and metastasis. Research has shown that terpenes exhibit anti-tumorigenic effects against these characteristics in multiple in vivo and in vitro studies, suggesting their potential application as chemotherapeutic agents for cancer treatment[155].

Numerous studies have shown the neuroprotective effects of essential oils obtained from various plants, including *O. gratissimum*, in both in vivo and in vitro environments. Therefore, terpenes, as key components of plant essential oils, may support human neuronal health[109]. However, limited attention has been given to the positive effects of terpene components from plant essential oils on brain health in research. To date, several terpenes produced by a diverse array of plants have been associated with neuronal well-being. One such terpene is borneol, a bicyclic monoterpene found in numerous medicinal plants[156].

2.9.4 Alkaloids

Alkaloids are compounds that are recognized for their ability to lower blood pressure and stabilize the nervous system in conditions affecting mental health. Alkaloids are also present due to the anti-malaria properties of certain plants' microbes. Several plants containing alkaloids may exhibit antibacterial properties, making them a valuable resource for traditional anti-malaria treatments. Alkaloids are considered important sources of medicinal plant compounds. Phytochemicals, which are natural substances found in plants, serve as medicines for human protection, exhibiting a variety of pharmacological effects on both humans and animals; examples include morphine, caffeine, and the alkaloids found in coffee, which are regarded as nitrogenous bases. These metabolites are well-established and their presence indicates that plants hold significant potential, as seen with tea and coffee being natural sources of alkaloids that stimulate the nervous system; many of these alkaloids have notable physiological effects on humans[155].

2.9.5 Steroids

Steroids are a category of secondary metabolites derived from cholesterol that possess a diverse range of chemical and biological properties[169]. The fundamental structure of a steroid molecule is a perhydroxyl cyclopentanophenanthrene, which is a common feature among nearly all steroid compounds. There are numerous types of steroids due to variations in the core structure and the addition of different functional groups. Ongoing research aims to explore these metabolites as potential lead compounds in drug design and discovery, as steroids have multiple therapeutic uses[170]. For example, ecdysteroids are polyhydroxy steroids produced by some plants, including those in the *O. gratissimum* family. Plants that contain ecdysterone display pharmacological properties such as anabolic, anti-diabetic, analgesic, anti-inflammatory, and anthelmintic effects[166].

2.10 Biological Activities of Scent Leaf (*Occimum gratissimum)*

2.10.1 Antioxidant Properties of Scent Leaf

Scent leaf's high levels of vitamins, such as alpha-tocopherol and ascorbic acid, are closely linked to its antioxidant capabilities. Previous research has shown that phenolic compounds and flavonoids help protect cells from damage caused by oxidative stress[172]. Flavonoids and phenols demonstrate their anti-inflammatory and antioxidant effects through various mechanisms, including scavenging free radicals, chelating metal ions, or inhibiting enzymatic pathways responsible for free radical generation. Herbal extracts have been utilized as alternative treatments for psychiatric issues associated with oxidative stress[173].

Some researchers employed LC-ESI-MS/MS to investigate the phytochemical components of a 70% ethanolic extract of *O. gratissimum* leaves (OGE), revealing a diverse array of substances, such as polyphenols, flavonoids, and fatty acids. The analysis showed that OGE contained flavonoids amounting to 25.33 ± 1.8 mg/g catechin equivalents and polyphenols at 124.3 ± 5.8 mg/g gallic acid equivalents. The antioxidant efficacy of *O. gratissimum* was assessed using in vitro assays, including DPPH, iron chelating, ABTS, nitric oxide, and hydroxyl radical scavenging, which yielded strong free radical scavenging activity with IC50 values of 470 ± 28.6, 330 ± 17.4, 133 ± 7.2, 83 ± 4.4, and 260 ± 12.7 µg/mL. OGE was also evaluated for its capacity to safeguard macromolecules such as DNA, proteins, and lipids from peroxidation damage[156].

Research indicates that *O. gratissimum* possesses antioxidant properties, protects against macromolecule damage, and has anxiolytic effects. Additionally, the plant is used in traditional medicine to treat various ailments, including upper respiratory infections, diarrhea, headaches, skin disorders, pneumonia, cough, fever, conjunctivitis, and gastrointestinal and gynecological issues. Both fresh and dried leaves of the plant contain terpenes, flavonoids, tannins, alkaloids, steroids, proteins, carbohydrates, fats, and oils, with higher concentrations found in the dried samples. Basil, which belongs to the same family as *O. gratissimum*, has been researched for its health benefits. Similar to *O. gratissimum*, basil is abundant in essential oils, phytochemicals, and nutrients, demonstrating antioxidant, anti-inflammatory, antimicrobial, and anticancer effects[155].

However, the specific makeup of these phytochemicals differs between basil and *O. gratissimum*. For instance, basil is particularly rich in phenols such as rosmarinic acid, known for its potent antioxidant and anti-inflammatory effects. Additionally, basil contains significant amounts of essential oils like eugenol, which exhibit antibacterial and antifungal properties. When comparing *O. gratissimum* and basil, both plants offer a variety of health benefits; however, the distinct compositions of their phytochemicals may enhance their effectiveness for particular conditions. *O. gratissimum*, for instance, has demonstrated anxiolytic properties that could potentially serve as a treatment for psychiatric disorders, while basil's high levels of rosmarinic acid may render it more effective for inflammation-related issues like arthritis. In summary, both *O. gratissimum* and basil are valuable sources of natural therapies with diverse potential health benefits[156].

2.10.2 Anti-Convulsant Properties of Scent Leaf

Research has identified that extracts from scent leaf exhibit anticonvulsant properties, as they delay the onset of seizures induced by PTZ and offer protection to treated mice against death related to seizures. PTZ induces convulsions by interfering with the chloride (Cl)-channel complex at the GABA A receptor, which reduces GABA-dependent inhibition. Based on a conceptual framework, the extracts and their fractions negatively impacted locomotor and exploratory behaviors, while positively affecting grooming and freezing behaviors in open-field tests related to depression and anxiety. Studies have noted that the leaves of *O. gratissimum* display significant anxiolytic-like characteristics. Research is ongoing to identify the anticonvulsant components[157].

2.10.3 Antimicrobial Properties of Scent Leaf

The phrase "antimicrobial activity" encompasses all active components that inhibit bacterial growth, prevent the proliferation of microbial colonies, and may even eliminate microorganisms. Various studies have validated the antimicrobial efficacy of *Ocimum gratissimum*, indicating that its essential oil serves as a plant-based preservative and suggesting its application as a non-toxic antibacterial and anti-aflatoxigenic agent against fungal contamination and aflatoxins in spices, as well as functioning as an antioxidant to prolong shelf life. Research has also revealed the existence of phytochemicals within the *O. gratissimum* plant, which exhibit substantial antimicrobial activities with minimal to no toxicity in laboratory animals. In contrast to conventional antimicrobials, which can result in hepatotoxicity when overdosed, the extract of *O. gratissimum* displays lower toxicity levels[158].

2.10.4 Anti-inflammatory Properties of Scent Leaf

It contains bioactive substances such as eugenol, thymol, and rosmarinic acid, which are known for their anti-inflammatory effects. These compounds may contribute to the reduction of pro-inflammatory cytokines and enzymes within the body, thereby alleviating inflammation. Furthermore, scent leaf possesses antioxidant qualities that can help mitigate oxidative stress, a significant factor in chronic inflammation. Overall, scent leaf may offer a beneficial natural solution for managing inflammation in the body [159].

2.10.5 Anti-Viral Properties of Scent Leaf

Scent leaf, also referred to as African basil, has been traditionally employed in herbal medicine for its potentially antiviral effects. It houses bioactive compounds, including eugenol, thymol, and rosmarinic acid, known to exhibit antiviral properties. These compounds might assist in inhibiting the replication of specific viruses, such as herpes simplex virus, influenza virus, and Human Immunodeficiency Virus (HIV). Additionally, scent leaf may support the immune system, enhancing the body's capacity to combat viral infections. Some research indicates that scent leaf may possess broad-spectrum antiviral activity, suggesting its effectiveness against a variety of viruses. While more investigation is required to thoroughly comprehend the antiviral properties of scent leaf, it could represent a promising natural treatment for viral infections[160].

2.10.6 Anti-fertility Effects of Scent Leaf

Scent leaf may exhibit potential antifertility attributes. It contains substances that could disrupt the balance of certain hormones in the body, including estrogen and progesterone, which are crucial for regulating the menstrual cycle and fertility. Moreover, scent leaf might negatively impact sperm function, hindering its capability to fertilize an egg. Although additional research is necessary to gain a comprehensive understanding of the antifertility implications of scent leaf, it might serve as a natural alternative for individuals aiming to reduce their fertility. It is important to emphasize, however, that scent leaf should not be relied upon as the sole method of contraception and that more dependable forms of birth control should be utilized[161].

2.10.7 Toxicity Effects of Scent Leaf

Scent Leaf, like all plants, can be toxic if used improperly. Individuals with allergies to plants from the mint or basil families should refrain from using fragrance leaves. If someone experiences hives, swelling, or difficulty breathing after eating food containing the leaves, they should call emergency services [161]. Additionally, Scent leaves are high in vitamin K, which plays a crucial role in blood clotting. Excessive intake of vitamin K from food or supplements can affect the efficacy of warfarin and other anticoagulants. Hence, those on blood thinners should consult a physician before significantly increasing their intake of fragrance leaf or any type of basil. A study in Nigeria investigated the use of high doses of scent leaf (*O. gratissimum*) leaf extract to stimulate labor in pregnant women and yielded promising results. Therefore, it is advisable for pregnant women to limit their intake of this herb to avoid the risk of premature labor, as it is not toxic for them[162]. The vitamins and antioxidants that are plentiful in scent leaf (*O. gratissimum*) contribute to disease prevention. Its essential oil is particularly rich in phytochemicals that help prevent colds, skin issues, bad breath, and microbial infections. However, pregnant women and those taking blood thinners should be cautious about consuming large amounts of fresh fragrance leaf extracts. The notion that natural plant-based supplements are completely safe and devoid of side effects is misleading until their properties have been exhaustively studied with various cellular or in vivo models[163].

O. gratissimum serves as an excellent functional component for addressing a broad range of health issues due to its remarkable nutritional and medicinal properties. Numerous researchers are currently conducting extensive investigations into the beneficial properties of this plant that enhance health. *O. gratissimum* is an exceptional source of essential phytochemicals. The most prevalent phytochemicals identified in Scent leaf include flavonoids, terpenes, tannins, and phenols. The synergy of these phytochemicals in scent leaf may contribute to its wide-ranging health benefits. Rich in nutrients and essential oils, bioactive extracts from this plant could offer a superior alternative to traditional medicine in treating conditions such as microbial infections, coughs, cancer, diarrhea, anemia, and inflammation[164]. While *O. gratissimum* shows potential for bioactivity, it also provides a rich source of micronutrients. The plant's biological properties are due to its variety of phytochemical constituents, which collaborate to deliver its health benefits. Pinpointing a single chemical component as the primary factor for the bioactivity of scent leaf is challenging, as it is the collective interaction of these components that generates its overall effects. The pharmacological and clinical effects of *O. gratissimum* have been found to extend beyond merely anti-inflammatory and antioxidant functions[165].

2.11 *Moringa oleifera*

Moringa is native to the Indian subcontinent and has become established in tropical and subtropical regions globally[166]. This family, Moringaceae, includes nearly thirteen Moringa species. For around 5000 years, Indians have incorporated it into their traditional diets. The Moringa tree can flourish in humid tropical climates or hot, arid regions, reaching an average height of 5 to 10 meters. It is resilient in extreme climates, tolerating poor soil conditions and drought effectively. It can adapt to a wide range of rainfall levels, from an estimated minimum of 250 mm to a maximum exceeding 3000 mm, and it thrives in a pH range of 5.0 to 9.0. Its trunk is soft, white, corky, and its branches have a gummy texture, with each compound leaf showcasing several small leaflets[167].

The flowers are white, and the three-winged seeds are carried by the wind. The flowers, tender leaves, and pods can be consumed as vegetables. The leaves are high in iron and are therefore highly recommended for expectant mothers. In various regions around the globe, *Moringa oleifera* is commonly called the 'drumstick tree' or the 'horseradish tree,' while in other places, it is known as kelor, marango, mlonge, moonga, mulangay, nebeday, saijhan, sajna, or the Ben oil tree. In India and Pakistan, Moringa is referred to as Sohanjna and is cultivated throughout the country. According to the Bureau of Plant Industry, *Moringa oleifera* is an exceptional source of nutritional elements. Its leaves (weight for weight) contain the same amount of calcium as four times that of milk, the vitamin C level is seven times greater than that found in oranges, while its potassium content is three times that of bananas, three times the iron content of spinach, four times the vitamin A found in carrots, and double the protein found in milk[162].

Figure 2.2: ***Moringa oleifera*** **Leaf**[160]

Moringa oleifera is also recommended as a valuable source of dietary minerals. The leaves and pods of Moringa are rich in Ca, Mg, K, Mn, P, Zn, Na, Cu, and Fe. However, the mineral composition of Moringa can vary depending on the geographical location. Many ancient medicinal traditions have utilized various plant products by traditional communities across different regions for treating diverse ailments. Among these plants, *Moringa oleifera* has played a significant role since ancient times. This plant possesses remarkable medicinal qualities that can address healthcare needs in numerous scenarios. Its easy cultivation even in challenging environmental conditions and its widespread availability make it appealing for both economic and health-related benefits, particularly in resource-limited developing countries[163].

2.11.1 Medicinal Properties of *Moringa oleifera*

Moringa's extensive medicinal properties have long been acknowledged in Ayurvedic and Unani medicine. Almost every component of this plant, encompassing the root, bark, gum, leaf, fruit (pods), flowers, seeds, and seed oil, has been utilized for treating various health issues in indigenous medicine. Recent studies, however, are also highlighting several active compounds that support its use in modern medicine[163]. *Moringa oleifera*, often referred to as the horseradish tree, drumstick tree, ben oil tree, miracle tree, and mother's best friend, is renowned for its multipurpose qualities, adaptability, and ease of growth. The leaves, pods, and flowers are rich in nutrients essential for both humans and animals. Moringa is one of the 14 species belonging to the family Moringaceae[163]. It is believed to have originally originated in the northern regions of India around 5000 years ago and subsequently spread to the southern parts, where it is known as 'Murungaikeerai' (*Moringa oleifera* leaves) and 'Murungakkai' (*Moringa oleifera* vegetable)[164]. Globally, *Moringa oleifera* has been utilized as traditional medicine for a variety of ailments, including anemia, skin infections, blackheads, anxiety, bronchitis, catarrh, chest congestion, asthma, blood impurities, cholera, glandular issues, swelling, headaches, conjunctivitis, cough, diarrhea, ear and eye infections, fever, hysteria, joint pain, pimples, psoriasis, respiratory issues, scurvy, semen deficiency, sore throat, sprains, tuberculosis, intestinal worms, lactation, diabetes, and during pregnancy. The medicinal properties of *Moringa oleifera* oil have been recognized by ancient cultures[165].

2.11.2 Health Benefits of *Moringa oleifera*

Moringa oleifera is classified within the Brassica order and belongs to the *Moringaceae* family, which contains a single genus with 13 known species[166]. This small tree, native to the sub-Himalayan regions of North West India, has become widespread across various regions in Africa, Arabia, South East Asia, the Pacific and Caribbean Islands, as well as South America. Traditionally, *Moringa oleifera* is not only a commonly consumed vegetable in these areas but is also recognized for its health-promoting properties, earning it the nickname 'the miracle tree' among the public due to its remarkable healing effects on a variety of ailments and some chronic conditions. Numerous studies have been conducted to extract bioactive compounds from different parts of the plant, which highlights its numerous applications. As a result, medicinal plants like *Moringa oleifera*, often referred to as phytomedicine, remain a reliable and widely used alternative in the field of medicine due to affordability[167].

Throughout history and across numerous cultures globally, *Moringa oleifera* has been employed medicinally to address issues such as skin infections, anemia, anxiety, asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera, and a host of other disorders[168]. This plant is known for its various activities, including anti-inflammatory, anti-spasmodic, anti-hypertensive, anti-tumor, antioxidant, anti-pyretic, anti-ulcer, anti-epileptic, diuretic, cholesterol-lowering, renal, anti-diabetic, and hepatoprotective properties. Additionally, it has long been recognized for its significant cosmetic benefits, with *Moringa oleifera* increasingly featured in numerous personal care products such as moisturizers and hair conditioners in recent years. Historical evidence suggests that *Moringa oleifera* oil has been utilized in skin ointments since ancient Egyptian times. *Moringa oleifera* is often regarded as 'the most nutrient-rich plant[169].'

2.11.3 Nutritional Composition of *Moringa oleifera*

The remarkable medicinal properties of *Moringa oleifera*, acknowledged by various cultures and communities through personal experiences, are gradually being validated by scientific research. Studies have revealed that *Moringa oleifera* is rich in essential nutrients such as vitamins, minerals, amino acids, beta-carotene, antioxidants, anti-inflammatory compounds, and omega-3 and omega-6 fatty acids. The nutritional composition of a plant is crucial in determining its medicinal, nutritional, and therapeutic attributes. It is considered that *Moringa oleifera* leaves are an excellent source of vitamin C, calcium, beta-carotene, potassium, and protein [170]. *Moringa oleifera* acts as a powerful source of natural antioxidants due to the presence of various antioxidant compounds, including flavonoids, ascorbic acid, carotenoids, and phenolic compounds, allowing it to prolong the shelf life of fats in food. Furthermore, studies conducted on different parts of the *Moringa oleifera* tree including fruits, seeds, leaves, flowers, bark, and roots have consistently revealed at least one or, in many instances, multiple beneficial nutrients. An article from the Trees for Life organization similarly stated that "every part of the *Moringa oleifera* tree is believed to possess beneficial properties that can aid humanity."

It is uncommon for a single plant to be abundant in numerous essential nutrients and, moreover, in significant amounts. Nonetheless, *Moringa oleifera* has been reported to have higher levels of various nutrients compared to those found individually in many other foods and vegetables. Research teams at the Asian Vegetable Research and Development Centre (AVRDC) pointed out that the leaves of four *Moringa oleifera* species were highly nutrient-rich and antioxidant-packed, with nutrient content influenced by factors such as preparation methods, leaf maturity, and harvest timing. As is widely recognized, most vegetables lose their nutritional value when cooked. However, it was found that *Moringa oleifera* leaves, whether fresh, cooked, or dried and stored for months at room temperature, retained their nutritional content. Boiling the leaves resulted in three times more bioavailable iron compared to raw leaves. Similar findings were observed with powdered Moringa leaves. Additionally, *Moringa oleifera* was found to contain a unique group of compounds consisting of sugar and rhamnose, which are rare sugar-modified glucosinolates. These compounds have been reported to exhibit certain chemopreventive effects by promoting apoptosis[171].

2.11.4 Antimicrobial and Antihelmintic Properties of *Moringa oleifera*

The antimicrobial properties of *Moringa oleifera* have been confirmed following the identification of its inhibitory effects against various microorganisms. A recent investigation demonstrated that aqueous extracts of *Moringa oleifera* (MO) exhibit inhibition against numerous pathogenic bacteria, including *Staphylococcus aureus, Bacillus subtilis, Escherichia coli*, and *Pseudomonas aeruginosa,* in a dose-dependent manner. Additionally, *Moringa oleifera* extracts showed inhibitory effects against *Mycobacterium phlei* and *B. subtilis*. The leaf extract of *Moringa oleifera* proved effective in preventing the growth of fungi such as *Basidiobolus haptosporus* and *Basidiobolus ranarum*. Another study evaluated the antimicrobial effects of aqueous methanolic extracts and fixed oils against various microorganisms, including *Scenedesmus obliquus* (a type of green algae), *E. coli* ATCC 13706, *P. aeruginosa* ATCC10145, *S. aureus* NAMRU 3 25923, *Bacillus stearothermophilus*, as well as Herpes Simplex virus type 1 (HSV 1) and Polio virus type 1 (sabin vaccine)[168].

The study found varying degrees of antimicrobial activity, with *B. stearothermophilus* being sensitive and *P. aeruginosa* showing resistance. In addition to antibacterial properties, MO oils also exhibit antifungal activity. A comparative study of the relative antimicrobial effectiveness of seed extracts against bacterial strains (*Pasteurella multocida, E. coli, B. subtilis*, and *S. aureus*) and fungal strains (*Fusarium solani* and *Rhizopus solani*) indicated that *P. multocida and B. subtilis* were the most susceptible strains, and their activity was influenced by cations (Na+, K+, Mg2+, and Ca2+)[169].

2.11.5 Anti-inflammatory Activity of *Moringa oleifera*

Various parts of the *Moringa oleifera* plant exhibit considerable anti-inflammatory effects. For example, the extract from its roots shows notable anti-inflammatory properties in rat paw oedema induced by carrageenan. The crude methanol extract from the roots suppresses carrageenan-induced paw oedema in a dose-dependent manner following oral administration. Additionally, the n-butanol extract derived from Moringa seeds demonstrates anti-inflammatory effects against ovalbumin-induced airway inflammation in guinea pigs. The powerful anti-inflammatory properties of *Moringa's oleifera* bioactive compounds may help alleviate inflammation-related chronic diseases. Given the significant anti-inflammatory effects of the *Moringa oleifera* plant, it can be inferred that this plant has a major impact on diseases linked to inflammation and the symptoms that arise from them. Therefore, it is believed that this plant may provide therapeutic benefits for conditions such as asthma, pain, and other related symptoms[169].

2.11.6 Multipurpose uses of *Moringa oleifera*

For decades, *Moringa oleifera* has been commonly utilized in the medical field to treat a wide range of acute and chronic ailments. In vitro investigations into the plant have suggested its potential for managing inflammation, hyperlipidemia, and hyperglycemia. The phytochemicals found in *Moringa oleifera*, including flavonols and phenolic acids, have been associated with anti-inflammatory, antioxidant, and antibacterial activities. Recently, studies have shifted focus towards food components that may trigger inflammatory responses, particularly due to evidence linking chronic low-grade inflammation with insulin resistance and obesity. Key biomarkers of inflammation have been recognized as critical indicators of the pro-inflammatory response seen in obesity-related diabetes, including cytokines such as tumor necrosis factor alpha (TNFa), interleukin-1 beta (IL-1b), interleukin-6 (IL-6), along with inducible nitric oxide synthase (iNOS), and nitric oxide (NO), which plays a vital role in insulin signaling and is catalyzed by iNOS. The overproduction of NO and iNOS expression have been associated with the development of disease states, particularly those connected to chronic inflammation. The hepatoprotective effects of Moringa seed extract, revealed through anti-fibrotic studies, indicate that *Moringa oleifera* also has anti-inflammatory properties against liver damage and fibrosis induced by CC14[170].

2.11.7 Antimicrobial Properties of *Moringa oleifera*

Various extracts from different parts of *Moringa oleifera*, including seeds cotyledon, seed coats, stem bark, leaves, and root bark, are noted for their antimicrobial properties. A recent study investigated the antimicrobial effectiveness of an aqueous extract from the pods' husks against both Gram-positive and Gram-negative pathogenic bacteria as well as yeast strains. The antimicrobial properties of *Moringa oleifera* were evaluated using the Kirby-Bauer disc diffusion method with a 50% ethanolic extract from *Moringa oleifera* leaves. The findings indicated that the 50% ethanolic extract exhibited some antibacterial properties, albeit minimal. Even at elevated concentrations, the extract showed only slight inhibitory effects and no activity against *Pseudomonas*. The antibacterial efficacy of the *Moringa oleifera* extract was more pronounced against Gram-positive species (*S. aureus and E. faecalis*) compared to Gram-negative species (*E. coli, Salmonella, P. aeruginosa, V. parahaemolyticus, and A. caviae*), a trend that has been corroborated by various other studies[171].

2.11.8 Anti-asthmatic Effects of *Moringa oleifera*

It has been noted for quite some time that the alkaloids found in the *Moringa oleifera* plant bear a resemblance to the action of ephedrine and can be utilized for asthma treatment. The alkaloid moringine is known to relax bronchial passages. Additionally, the seed kernels of *Moringa oleifera* demonstrated a beneficial effect in managing bronchial asthma, as evidenced in a study focused on the effectiveness and safety of seed kernels for asthmatic patients. The results revealed a notable reduction in asthma symptoms and an improvement in respiratory function[172].

2.11.9 Analgesic Properties of *Moringa oleifera*

The analgesic properties of various *Moringa oleifera* species have been documented in several studies. In research utilizing ethanolic extracts of the tender pod-like fruits of *Moringa oleifera* concanensis in experimental animals, significant analgesic effects were observed. Moreover, alcoholic extracts derived from the leaves and seeds of *Moringa oleifera* also exhibited substantial analgesic effects, as demonstrated through the hot plate and tail immersion methods [173].

2.11.10 Antipyretic Properties of *Moringa oleifera*

Based on the anti-inflammatory effects of *Moringa oleifera's* bioactive components, one can hypothesize its antipyretic effects. A study was designed to evaluate the antipyretic effects of ethanol, petroleum ether, solvent ether, and ethyl acetate extracts from *Moringa oleifera* seeds using a yeast-induced hyperpyrexia model. Paracetamol served as the control in this study.

Unsurprisingly, both ethanol and ethyl acetate extracts from the seeds demonstrated significant antipyretic effects in rats.

Antihypertensive, diuretic, and cholesterol-lowering effects of *Moringa oleifera* are attributed to its numerous bioactive compounds, which directly influence blood pressure, making them beneficial for blood pressure regulation. Compounds such as nitrile, mustard oil glycosides, and thiocarbamate glycosides found in *Moringa oleifera* leaves contribute to the blood pressure-lowering effects. Additionally, the roots, leaves, flowers, gum, and the aqueous infusion of seeds also possess diuretic properties. Furthermore, *Moringa oleifera* leaves contain the bioactive phytoconstituent b-sitosterol, which is known for its ability to lower cholesterol levels in the serum of rats on a high-fat diet [174].

2.11.11 Antidiabetic Activity of *Moringa oleifera*

A variety of medicinal plants have been assessed for their therapeutic capabilities in treating diabetes, and *Moringa oleifera* is a significant contender in this field. Research has shown that *Moringa oleifera* leaves considerably reduce blood glucose levels in Wistar rats and Goto-Kakizaki (GK) rats, which are models for type 2 diabetes. Another investigation revealed that the extract from *Moringa oleifera* leaves can lower blood sugar levels within three hours after ingestion. As an explanation for *Moringa oleifera's* antidiabetic effects, it has been suggested that polyphenols from dark chocolate and other sources are linked to its hypoglycemic properties. *Moringa oleifera* leaves are a rich source of polyphenols, including quercetin-3-glycoside, rutin, kaempferol glycosides, and various other polyphenols. Therefore, the potential antidiabetic properties of *Moringa oleifera* could be harnessed for commercialization through the development of appropriate technologies that achieve efficacy comparable to conventional medications.

2.11.12 Antioxidant Activity of *Moringa oleifera*

Moringa oleifera is noted for being a plentiful source of antioxidants. Research has demonstrated that aqueous extracts from the leaves, fruits, and seeds of *Moringa oleifera* function as antioxidants. The methanol and ethanol extracts from Indian *Moringa oleifera* show the highest antioxidant activities, with percentages of 65.1 and 66.8%, respectively. It has also been established that significant bioactive compounds, such as phenolics, including quercetin and kaempferol, contribute to these antioxidant effects. In another study, quercetin and kaempferol displayed notable antioxidant activity regarding hepatocyte growth factor (HGF) induced Met phosphorylation, with IC50 values of 12 and approximately 6 μM/L, respectively. *Moringa oleifera* seeds were found to be superior in radical scavenging potential when compared to palm oil. Naturally occurring antioxidants, particularly polyphenols, are key plant compounds that can reduce oxidative damage in cells through indirect cell enhancement or by free radical scavenging. *Moringa oleifera* tree leaves have been identified as exhibiting antioxidant properties due to their high polyphenol content. Extracts from both mature and young Moringa leaves demonstrate strong antioxidant activities against free radicals, helping to prevent oxidative damage to major biomolecules and offering significant protection against oxidative injury[176].

2.11.13 Hepatoprotective Activity of *Moringa oleifera*

Numerous studies have revealed that *Moringa oleifera* exhibits considerable hepatoprotective properties [178]. Ethanol extracts of *Moringa oleifera* leaves demonstrated notable protection against liver damage caused by antitubercular medications such as isoniazid (INH), rifampicin (RMP), and pyrazinamide (PZA) in rats. The hepatoprotective effects of Moringa are mediated by its influence on serum levels of glutamic oxaloacetic transaminase (aspartate aminotransferase), glutamic pyruvic transaminase (alanine aminotransferase), alkaline phosphatase, and bilirubin, as well as on lipid levels and lipid peroxidation in the liver.

Additionally, methanol and chloroform extracts of *Moringa oleifera* leaves showed marked protection against liver damage induced by CCl4 in albino rats[179]. Beyond the protective attributes of *Moringa oleifera* leaves, both its roots and flowers also exhibit significant hepatoprotective effects. The flowers of *Moringa oleifera* contain a well-known flavonoid, Quercetin, which may contribute to its potent liver-protective effects. A recent investigation into the effects of *Moringa oleifera* seed extract on liver fibrosis indicated that the seed extract can help alleviate liver fibrosis. In this study, CCl4 was used to induce liver fibrosis, with concurrent administration of *Moringa oleifera* seed extract showing that it regulated the rise in serum aminotransferase activities and globulin levels prompted by CCl4. Furthermore, immunohistochemical analyses revealed that *Moringa oleifera* reduces liver fibrosis[178].

Moringa has been identified as a potent source of anticancer activity, driven by several bioactive compounds. Niazimicin is also reported to inhibit tumor promoter teleocidin B4-induced activation of Epstein-Barr virus (EBV). In another study examining 11 plants used in traditional Bangladeshi medicine, *Moringa oleifera* was highlighted as a promising source of anticancer agents[179]. The investigation also suggested the potential cytotoxic effects of *Moringa oleifera* leaf extract on human multiple myeloma cell lines. In addition to the leaves, *Moringa oleifera* seed extracts also exhibit anticancer properties through their effects on hepatic carcinogen metabolizing enzymes and their antioxidant characteristics[180].

2.16.9 Antifertility Activity of *Moringa oleifera*

The *Moringa oleifera* plant is also noted for its relevant antifertility effects; the aqueous extract derived from the roots and bark has shown post-coital antifertility impacts in rats and has induced fetal resorption during late pregnancy. The aqueous extract of Moringa roots was assessed for its estrogenic, anti-estrogenic, progestational, and antiprogestational properties. This extract produces several effects that contribute to its antifertility capability[181]. In a different study reviewing the anti-reproductive effects of plants used in folk medicine, *Moringa oleifera* leaf extracts were determined to be 100% abortive at doses equivalent to 175 mg/kg of the initial dry material[181].

2.11.15 Cardiac and Circulatory Stimulator from *Moringa oleifera*

Beyond the previously mentioned bradycardiac effects of *Moringa oleifera* leaves, it has been reported that all parts of *Moringa oleifera* exhibit some cardiac and circulatory stimulant properties[182]. The root bark of *Moringa oleifera* contains the alkaloid moringinine, which functions as a cardiac stimulant by influencing the sympathetic nervous system. These effects might also be attributed to the prevention of hyperlipidemia. Research has demonstrated that *Moringa oleifera* is effective in preventing hyperlipidemia in iron-deficient male Wistar rats [183]. In a study that compared the effects of Moringa leaf extract to atenolol (a selective β1 receptor antagonist used for treating cardiovascular conditions), parameters such as serum cholesterol, triglycerides, blood glucose levels, heart weight, and body weight were evaluated in rats induced with adrenaline. The findings indicated that *Moringa oleifera* leaf extract resulted in notable alterations in cardiovascular parameters. This research identified Moringa leaf extract as having hypolipidaemic properties, reducing body weight, heart weight, levels of serum triglycerides, and serum cholesterol in the test animals. Furthermore, additional studies explored the antiatherosclerotic and hypolipidaemic effects of *Moringa oleifera* leaves using simvastatin as a control. *Moringa oleifera* has also been shown to provide cardio protective benefits in female Wistar albino rats experiencing myocardial infarction induced by isoproterenol (ISP). It was reported that treatment with *Moringa oleifera* positively affects various biochemical enzymatic parameters, including superoxide dismutase, catalase, glutathione peroxidase, lactate dehydrogenase, and creatine kinase-MB. Additionally, it helps to prevent histopathological damage and alterations in ultra-structure caused by ISP-induced myocardial infarction[184].

2.12 Enteric Bacteria

Enteric bacteria are a diverse group of Gram-negative, facultatively anaerobic rods that inhabit the intestinal tracts of humans and other animals[185]. These bacteria are commonly found in the family *Enterobacteriaceae*, which includes many genera such as *Escherichia*, *Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter*, *Proteus*, and *Yersinia*. While some enteric bacteria are harmless commensals that contribute to the normal gut flora and aid in digestion, others are pathogenic and responsible for a wide range of gastrointestinal and systemic infections[186].

Enteric bacteria are primarily characterized by their ability to ferment glucose, their presence of peritrichous flagella (if motile), and their negative result in the oxidase test. They typically reside in the lower gastrointestinal tract, where they play essential roles in maintaining gut homeostasis, producing certain vitamins such as vitamin K, and preventing colonization by pathogenic microorganisms through competitive inhibition[187]. One of the most well-known enteric bacteria is *Escherichia coli* (E. coli), a versatile organism that exists in both harmless and pathogenic forms. Non-pathogenic strains are part of the normal flora and are vital for nutrient absorption and immune system development. However, certain pathogenic strains such as enterotoxigenic E. coli (ETEC), enterohemorrhagic E. coli (EHEC), and enteropathogenic E. coli (EPEC) can cause severe gastrointestinal diseases including diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome[188].

Salmonella species, especially *Salmonella enterica*, are important enteric pathogens responsible for foodborne illnesses. They cause diseases ranging from mild gastroenteritis to more serious systemic infections such as typhoid fever, primarily caused by *Salmonella Typhi*[189]. These bacteria are typically transmitted through the ingestion of contaminated food or water and can survive harsh conditions, including stomach acidity, allowing them to reach and infect the intestines. *Shigella* is another genus of enteric bacteria that causes bacillary dysentery or shigellosis. This condition is marked by severe abdominal cramps, fever, and bloody diarrhea. *Shigella* species are highly infectious, with a low infectious dose, and spread easily through person-to-person contact or via contaminated food and water[190].

Klebsiella species, particularly *Klebsiella pneumoniae*, can cause serious infections in both the gastrointestinal tract and other body systems such as the lungs and urinary tract. While often part of the normal flora, they can become opportunistic pathogens in immunocompromised individuals, leading to conditions like pneumonia, bloodstream infections, and liver abscesses[191]. *Proteus* species, known for their strong urease activity and swarming motility on culture media, are typically involved in urinary tract infections but can also affect the gastrointestinal system. Their ability to alkalinize urine by breaking down urea contributes to the formation of kidney stones. *Yersinia* enterocolitica is an enteric bacterium associated with foodborne gastroenteritis, especially in colder climates. It can mimic appendicitis and often arises from the consumption of undercooked pork products. A more notorious member of the *Yersinia* genus is *Yersinia pestis*, which causes plague, though it is not typically considered an enteric pathogen[192].

Enteric bacteria and *Staphylococcus* are distinct groups of bacteria, but they can share certain environments, cause overlapping clinical conditions, and pose similar public health concerns. Although they differ in taxonomy, morphology, and primary habitat, their relationship becomes relevant in the context of human health, infection control, and antimicrobial resistance. Enteric bacteria, primarily from the family *Enterobacteriaceae*, inhabit the gastrointestinal tract and include genera such as *Escherichia*, *Salmonella*, *Shigella*, and *Klebsiella*[193]. These bacteria are Gram-negative rods and are typically associated with intestinal infections, foodborne illnesses, and opportunistic infections such as urinary tract infections and sepsis. *Staphylococcus*, particularly *Staphylococcus aureus*, is a Gram-positive coccus that is part of the normal flora of the skin, nose, and mucous membranes. It is not an enteric bacterium by nature, but it can be found transiently in the gastrointestinal tract and may sometimes be isolated from stool samples, especially in individuals who are colonized or infected[194].

Staphylococcus is a genus of Gram-positive bacteria that are spherical in shape and often form clusters resembling bunches of grapes when viewed under a microscope. These bacteria are non-motile, non-spore-forming, and facultative anaerobes, meaning they can grow in both the presence and absence of oxygen[195]. *Staphylococci* are widely distributed in the environment and are commonly found on the skin and mucous membranes of humans and animals. The most clinically significant species within this genus is *Staphylococcus aureus*, known for its ability to cause a wide range of infections, from superficial skin infections to life-threatening systemic diseases. *Staphylococcus aureus* is distinguished by its production of coagulase, an enzyme that causes blood to clot, and this property is often used to differentiate it from other less pathogenic staphylococcal species, collectively referred to as coagulase-negative staphylococci. Another important feature of *S. aureus* is its golden pigment, which gives colonies a yellowish appearance on agar media[196].

S. aureus is a common cause of skin and soft tissue infections such as boils, impetigo, cellulitis, and abscesses. It can also invade deeper tissues, leading to serious conditions like pneumonia, osteomyelitis, septicemia, endocarditis, and toxic shock syndrome. One of the major concerns with *S. aureus* is its ability to produce a variety of virulence factors, including toxins, enzymes, and surface proteins that facilitate adhesion, immune evasion, and tissue destruction[197]. Notable among these toxins are the toxic shock syndrome toxin-1 (TSST-1), exfoliative toxins responsible for scalded skin syndrome, and enterotoxins that cause staphylococcal food poisoning. The rise of antibiotic-resistant strains of *Staphylococcus aureus* has become a significant global health concern. Methicillin-resistant *Staphylococcus aureus* (MRSA) is resistant to beta-lactam antibiotics and is a leading cause of hospital-acquired and community-acquired infections. MRSA infections are more difficult to treat and require the use of alternative antibiotics such as vancomycin, linezolid, or newer agents like daptomycin. Vancomycin-intermediate and vancomycin-resistant strains have also emerged, further complicating treatment efforts[198].

Coagulase-negative staphylococci, such as *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, and others, are part of the normal flora of the skin and mucous membranes. Although generally less virulent than *S. aureus*, these species can cause infections, especially in immunocompromised individuals or when medical devices such as catheters, prosthetic joints, or heart valves are in place. *S. epidermidis* is notorious for forming biofilms on the surfaces of medical devices, which protect the bacteria from antibiotics and the immune system, making infections persistent and difficult to eradicate. *Staphylococcus saprophyticus* is a common cause of urinary tract infections, particularly in young women[199]. Laboratory diagnosis of staphylococcal infections involves Gram staining, culture on selective media such as mannitol salt agar, and biochemical tests to identify coagulase production. Molecular methods, such as PCR and nucleic acid amplification tests, are increasingly used for rapid and accurate identification, especially in critical care settings[200].

The relationship between these groups emerges in several important clinical and epidemiological scenarios. Both can be involved in nosocomial (hospital-acquired) infections and are known for their ability to develop resistance to multiple antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL)-producing enteric bacteria are examples of multidrug-resistant organisms that pose significant treatment challenges in healthcare settings. In some cases, patients may be colonized or infected with both types of organisms simultaneously, particularly in immunocompromised or critically ill individuals[201].

Additionally, both enteric bacteria and *Staphylococcus aureus* can cause foodborne illness, although through different mechanisms. *S. aureus* causes food poisoning through the production of heat-stable enterotoxins in improperly stored food. In contrast, enteric pathogens such as *Salmonella* or *E. coli* typically cause illness through direct infection of the gastrointestinal tract after ingestion of contaminated food or water[202]. Infections caused by either group can lead to similar systemic complications such as bacteremia, septic shock, and organ dysfunction, particularly when the pathogens enter the bloodstream or are involved in polymicrobial infections. In certain surgical procedures, both types of bacteria are relevant considerations in perioperative infection prevention strategies. From a microbiological perspective, both are studied for their ability to acquire resistance genes, their interaction with the human immune system, and their adaptation to various environments. The gastrointestinal tract, particularly when disrupted by antibiotics, can serve as a reservoir for both enteric bacteria and *Staphylococcus aureus*, facilitating colonization and transmission[203].

Diagnosis of infections caused by enteric bacteria usually involves stool cultures, biochemical tests, antigen detection, and molecular methods like polymerase chain reaction (PCR). In clinical settings, differentiating these bacteria is critical for proper treatment and epidemiological tracking[204]. Selective media such as MacConkey agar, XLD agar, and EMB agar are often used to isolate and identify enteric organisms based on their lactose-fermenting capabilities and colony morphology. Antibiotic resistance among enteric bacteria is a growing concern worldwide, especially in nosocomial infections and among multidrug-resistant strains. The misuse and overuse of antibiotics have led to the emergence of extended-spectrum beta-lactamase (ESBL)-producing strains and carbapenem-resistant Enterobacteriaceae (CRE), posing significant challenges to treatment and public health[205].

Preventing *staphylococcal* infections requires good hygiene practices, proper wound care, sterilization of medical equipment, and strict infection control measures in healthcare facilities. Screening and decolonization strategies may be employed in hospitals to reduce the risk of MRSA transmission[206]. Vaccines targeting *S. aureus* are under development, though none have been approved for general use as of yet. *Staphylococcus* is a medically important genus of bacteria that includes both harmless commensals and aggressive pathogens. The ability of *S. aureus* to cause diverse infections, coupled with its adaptability and resistance to multiple antibiotics, makes it one of the most challenging bacterial pathogens in modern medicine[207].

In public health terms, controlling infections caused by pathogenic enteric bacteria involves improving sanitation, ensuring food and water safety, promoting good hygiene practices, and employing appropriate vaccination strategies, such as the typhoid vaccine[208]. Surveillance systems and infection control measures are essential to track outbreaks and prevent the spread of resistant strains. Enteric bacteria represent a complex group of organisms with significant roles in both health and disease[209]. While many are essential for normal gastrointestinal function, others are formidable pathogens responsible for major public health challenges. Understanding their biology, modes of transmission, pathogenic mechanisms, and resistance patterns is crucial for effective clinical management and prevention strategies[210].

Endnotes

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Chapter Three

Methodology

3.1 Collection of Plant Materials, Identification and Processing

Mature fresh leaves of *O. gratissimum* (Scent leaf) and *M. oleifera* were harvested. Identified by Mr Donatus Esimekhuai P.O with Identification numbers: UIH – 23604 and UIH – 23607 respectively at the Department of Botany, University of Ibadan. Harvesting was done between the hours of 16:00 and 17:00 when the plants must have completed their light stage of photosynthetic process for the day. The quantity of leaves needed was air dried at an ambient temperature of 27°C for two weeks and then milled with a hammer mill sieve of 0.02 mm pore size to obtain a fine powdery dust. The powdered test samples were stored in a dry, clean container with lid for further analysis.

3.2 Collection of the Test Organisms

Pathogenic strains of *Escherichia coli* (ATCC 25922) *Klebsiella pneumonia*, *Salmonella typhi*, *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* were obtained from the Department of Medical Microbiology and Parasitology, University College Hospital (UCH) Ibadan, Nigeria.

3.3 Preparation of Plant Extracts

The dried and powdered leaf samples were subjected to reflux extraction in a Soxhlet apparatus for twelve hours, allowing for successive extraction using organic solvents of varying polarity. Methanol, n-hexane, chloroform, and water served as the solvents, ranging from non-polar to polar. The obtained extracts were vacuum-dried and stored in sterile containers under refrigeration. Prior to experimentation, all phytochemical extracts, except for the aqueous extract, were dissolved in dimethyl sulfoxide (DMSO), while the water extract was dissolved in distilled water.

Proximate Analysis

Moisture content was determined by drying the samples in an oven at 105°C until a constant weight was achieved. For total ash content, the leaf samples were weighed and incinerated in a muffle furnace at temperatures of 450°C and 550°C. Crude fat content was assessed through extraction with hexane using a Soxhlet apparatus, following the standard procedures outlined by AOAC. The crude protein content was analyzed using the Kjeldahl method. Carbohydrate content was estimated by subtracting the sum of all other proximate components from 100%. The energy values were calculated by applying the Atwater conversion factors, multiplying carbohydrate, protein, and fat by 17, 17, and 37, respectively[1].

3.4 Phytochemical Screening of the Extracts of the *Moringa oleifera* and *Occium graticimmum*

3.4.1 Qualitative Determinations Procedure

Phytochemical investigation was carried out on the plant extracts for both qualitative and quantitative testing with the solvent extract of the plant using standard procedures to identify the bio-active constituents in which method used for the quantitative analysis as described[2].

a. Test for Tannins

Approximately 0.5 g of the dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride solution were added to the filtrate, and the presence of tannins was confirmed by the appearance of a brownish-green or blue-black coloration[3].

b. Test for Phlobatannins

The presence of phlobatannins was determined by boiling an aqueous extract of the plant sample with 1% hydrochloric acid. The formation of a red precipitate indicated a positive result[3].

c.* Test for *Saponins

Two grams (2 g) of the powdered sample was boiled in 20 ml of distilled water using a water bath and then filtered. A volume of 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously to observe the formation of a stable, persistent froth. To further confirm the presence of saponins, three drops of olive oil were added to the frothing solution, shaken vigorously, and examined for the formation of an emulsion[3].

d. Test for Flavonoids

Three different methods were employed to detect flavonoids in the plant sample:

1. Five (5) ml of dilute ammonia solution was added to a portion of the aqueous filtrate, followed by the addition of concentrated sulfuric acid (H_2SO_4). A yellow coloration that faded upon standing indicated the presence of flavonoids.
2. A few drops of 1% aluminum chloride solution were added to another portion of the filtrate, and the appearance of a yellow coloration confirmed the presence of flavonoids.
3. A portion of the powdered plant sample was heated with 10 ml of ethyl acetate over a steam bath for three minutes. After filtration, 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution, and the appearance of a yellow coloration was taken as a positive test for flavonoids[4].

e. Test for Steroids

To detect steroids, 2 ml of acetic anhydride was added to 0.5 g of the ethanolic extract, followed by 2 ml of concentrated sulfuric acid (H_2SO_4). A color change from violet to blue or green indicated the presence of steroids.

f. Test for Terpenoids (Salkowski Test)

A volume of 5 ml of the plant extract was mixed with 2 ml of chloroform. Concentrated sulfuric acid (3 ml) was carefully added to form a separate layer. The presence of terpenoids was confirmed by the appearance of a reddish-brown coloration at the interface[5].

g. Test for Anthraquinones

Two hundred milligrams (200 mg) of the powdered sample was boiled with 6 ml of 1% hydrochloric acid and then filtered. The filtrate was shaken with 5 ml of benzene and filtered again. Subsequently, 2 ml of 10% ammonia solution was added to the filtrate and shaken. The appearance of a pink, violet, or red coloration in the ammoniacal phase indicated the presence of free hydroxyl anthraquinones[6].

h. Test for Carbohydrates

Three (3) ml of the sample solution was placed in a clean, dry test tube. A few drops of α-naphthol solution were added and the mixture was gently shaken. Finally, 1 ml of concentrated sulfuric acid (H_2SO_4) was carefully added along the inner wall of the test tube and allowed to stand for a few minutes. The formation of a violet or purple ring at the junction of the two liquids confirmed the presence of carbohydrates[7].

3.4.2 Quantitative Determinations Procedure

Determination of Total Phenolic Content

a. Preparation of Gallic Acid Standard

A stock solution was prepared by dissolving 0.5 g of gallic acid in 10 ml of ethanol and then diluting the solution to 100 ml with distilled water, resulting in a final concentration of 5 g/L. Standard solutions of 50, 100, 250, and 500 mg/L were prepared by diluting 1, 2, 5, and 10 ml of the stock solution to a final volume of 100 ml with water. These standards were stored at 4°C for up to two weeks. When stored in a sealed container under refrigeration (4°C), the standards retain approximately 98% of their potency for up to two weeks, but only for five days at room temperature. Commercially available gallic acid is typically of sufficient purity; however, if needed, it can be recrystallized from water[8].

b. Preparation of Sodium Carbonate Solution (Na_2CO_3)

Ten grams (10 g) of anhydrous sodium carbonate was dissolved in 800 ml of distilled water and brought to a boil. After cooling, the solution was filtered using Whatman No. 1 filter paper and diluted to a final volume of 1 liter with water.

Sample Extraction

One gram (1 g) of the powdered plant sample was weighed into a flask. Twenty-five (25) ml of 70% methanol was added, and the mixture was vigorously shaken for ten minutes before being filtered. A 0.5 ml aliquot of the filtrate was then taken for analysis.

Analysis Procedure

A 0.5 ml sample was pipetted into a 50 ml volumetric flask, and 35 ml of distilled water was added. The same procedure was followed for the standard solutions. Then, 2.5 ml of Folin-Ciocalteu (FC) reagent was added to each flask, and the mixture was swirled and incubated at room temperature for one to eight minutes. Afterward, 7.5 ml of sodium carbonate solution was added, and the final volume was adjusted to 50 ml. The solution was mixed thoroughly and incubated for two hours at room temperature. The absorbance of the samples was measured at 765 nm using a spectrophotometer. If a sample exhibited an absorbance higher than that of the 500 mg/L standard, it was diluted accordingly and remeasured[9].

The total phenolic content was expressed as mg of gallic acid equivalent (GAE) per gram of dry extract. The concentration of total phenolic compounds in the extract was determined using the formula:

T=CVMT = \frac{CV}{M}T=MCV

Where:

i. **T** = Total phenolic content (mg/g of plant extract in GAE)
ii. **C** = Concentration of gallic acid from the calibration curve
iii. **V** = Volume of the extract in ml
iv. **M** = Mass of the pure plant extract (g)

c. Determination of Terpenoids

A sample of 100 mg (initial weight, W_i) of dried plant extract was soaked in 9 ml of ethanol for 24 hours, followed by filtration through Whatman filter paper. The filtrate was then extracted with 10 ml of petroleum ether using a separating funnel. The ether extract was collected separately and evaporated completely in pre-weighed glass vials (final weight, Wf). The total terpenoid content was determined using the following formula:[10.]

\text{Terpenoid Yield (%) } = \frac{(Wf - W_i)}{W_i} \times 100

d. Saponin Determination

Procedure

One (1) g of the sample (W_1) was weighted into a flask. De-fatted by adding 30ml of n-hexane Thirty (30) ml of methanol was added to the residue and filtered. Another thirty (30) ml of methanol was added to the residue and filtered. The concentrate or filtrate was heated to one-quarter (1/4) and 100ml of cold acetone was added. It was put in the fridge for fifty(50) mins. Empty filter paper was weighted as (W_2). It was dried in the oven at 30 – 40^{o}c and allowed to cool and the filter paper was re weighted after drying (W_3)[11].

$$\% \text{ Saponin} = \frac{W_3 \text{ x } W_2}{W_1} \text{ x } \frac{100}{1}$$

Where W_1 = Weight of sample

W_2 = Weight of empty filter paper

W_3 = Weight of filter + residue after drying

e. Alkaloid Determination

Procedure

Five (5) g of finely ground powered sample was weighted into a flask. 50ml of 10% acetic acid was added in ethanol (10% acetic acid in ethanol is ten (10) ml of acetic acid into ninety (90) ml of ethanol. It was allowed to stand for 4hrs at 28^{o}c and filtered. The filtrate was heated in a hot plate to one-quarter of its original volume by evaporation. The remaining concentrate was added with drop wise addition of 5ml (one ammonium hydroxide solution until all the alkaloid was precipitated. Filter paper (W_2) was weighted and filtered the precipitate through the pre-weighed filter paper. the filter paper washed while still inside the funnel with 1% ammonia solution. It was dried in the oven at 30^{o}c, allowed to cool and weighed the dried filter paper as (W_3). Alkaloid content was calculated[12].

$$\% \text{ Alkaloid} = \frac{W_3 \text{ x } W_2}{\text{Wt of sample } (W_1)} \text{ x } \frac{100}{1}$$

f. Determination of Cyanogenic Glycoside

Cyanogenic glycoside quantitative determination methodology was used in this research. It was weighed into a 250 cm3 round bottom flask and about 200 cm3 of distilled water was added to one gram of each dry wood powder sample and allowed to stand for 2 hours for autolysis to occur[6]. Full distillation was carried out in a 250 cm3 conical flask containing 20 cm3 of 2.5% NaOH (sodium hydroxide) in the sample after adding an antifoaming agent (tannic acid). Cyanogenic glycoside (100 cm3), 8 cm3 of 6 M NH4OH (ammonium hydroxide), and 2 cm3 of 5% KI (potassium iodide) were added to the distillate(s), mixed, and titrated with 0.02 M AgNO3 (silver nitrate) using a microburette against a black background. Turbidity which was continuous indicates the end point[13].

Content of cyanogenic glycoside in the sample was calculated as

$$\text{Cyanogesic Glycoside (mg/100g)} = \frac{\text{Titre value (cm3) x 1.08 x exact volume}}{\text{Aliquot vol x samplw wt}} \text{x100}$$

g. Tannin Determination

Apparatus

Reagent

17% sodium carbonate (Na_2C0_3), Folin Denis, Tannin standard stock solution (100ppm), Dissolve 0.1g of tannic acid salt was dissolved in one thousand (1000) ml volume flask and made up to mark with distilled H_20, 0.1g of tannic acid = 100ml (1 Litre) = 100ppm[14].

h. Tannin Working Standard (10ppm)

Ten (10) ml was pipetted from the stock one hundred (100)ppm) into one hundred (100) ml volume flask and made up to the mark. From this 10ppm, 0ppm, 2ppm, 4ppm, 6ppm, 8ppm & 10ppm were prepared into fifty (50) ml volume flask pipetting 0ml, 1ml, 2ml, 3ml, 4ml & 5ml respectively into fifty (50) ml volume flask.

Procedure

0.2g of finely grounded sample was weighted and added twenty (20) ml of 50% methanol. Covered with paraffin and placed in a water bath at 80^{o}c for one (1) hour and allowed to cool and filtered the extraction using whatman filter paper. It was filtered into a 100ml volume flask and make up the filtrate with distilled H_2O to the 100ml mark. 1ml of the extract or filtrate was filtered into fifty (50) ml volume flask. Twenty (20) ml of distilled H_20 was added to sample and standard .2.5ml folin – Dennis reagent was added to sample and standard. Ten (10) ml of 17% sodium carbonate solution was added to sample and standard and mixed properly and made up to mark with distilled H_2O .It was allowed to stand for 20minutes for a bluish–green colour to develop. Read absorbance at 760nm

$$\%\ \text{Tannin} = \frac{\text{Abs x Average Gradient x D. F}}{\text{Wt of sample x 1000}}$$

Where D.F. = Dilution factor

3.5 Biochemical Tests Identification of Tested Microorganisms

3.5.1 Gram Staining

3.5.2 Gram's Staining Procedure

Thin smear of pure bacterial culture were made on clean glass slide air dried and heat fixed. Smear was covered with crystal violet for 30 seconds, washed with distilled water and smear was flooded with Grams iodine solution for 60 seconds, washed with 95% ethyl alcohol and then distilled water again, the smear will be covered with safranin for 30 seconds, wash with distilled water and blot dried. Air dried and observed under microscope[15].

3.5.3 Catalase Test

The catalase test is used to differentiate microorganisms based on their ability to produce the enzyme catalase. This enzyme breaks down hydrogen peroxide (H_2O_2) into water and oxygen ($2H_2O_2 \rightarrow 2H_2O + O_2$), thereby detoxifying it. The test was performed under a laminar flow hood by mixing a small inoculum of the bacterial isolate with two to three drops of 3% hydrogen peroxide solution. The presence of catalase activity was indicated by the rapid formation of oxygen bubbles, while the absence or weak production of bubbles signified a negative result[16].

3.5.4 Indole Test

The indole test is significant for classifying and identifying anaerobic bacteria by assessing their ability to degrade the amino acid tryptophan and produce indole. Certain bacteria express the enzyme tryptophanase, which catalyzes the deamination and hydrolysis of tryptophan.

A 24-hour-old bacterial culture was inoculated into 10 mL of tryptophan broth, which had been previously sterilized at 121°C for 15 minutes and allowed to cool before inoculation. The broth was then incubated at 37°C for 24–48 hours. After incubation, 5 drops (0.5 mL) of Kovac's reagent were added to the culture, gently shaken, and left to stand for 20 minutes. A deep red color formation at the surface of the broth indicated a positive result, whereas a yellow coloration signified a negative result[17].

3.5.5 Citrate Test

The citrate test determines whether an organism can utilize citrate as its sole source of carbon and energy. To prepare the medium, 24 g of citrate agar was dissolved in 500 mL of distilled water and sterilized using an autoclave. The sterilized medium was then dispensed into Petri dishes and allowed to solidify. Bacterial isolates were aseptically inoculated by streaking once across the agar surface. The plates were incubated for 24 to 48 hours, after which color changes were observed. A shift from green to blue indicated a positive result (citrate utilization), while retention of the green color signified a negative result[18].

3.5.6 Starch Hydrolysis Test

This test was conducted to identify bacterial isolates capable of hydrolyzing starch, which consists of amylose and amylopectin. Starch agar plates were inoculated with the test bacteria and incubated at 35°C for 48 hours. After incubation, the plates were flooded with an aqueous iodine solution for 30 seconds. Iodine reacts with starch, producing a dark brown coloration. A clear zone surrounding the bacterial growth indicated a positive result (starch hydrolysis), while a blue-black coloration suggested a negative result[19].

3.5.7 Oxidase Test

An oxidase test strip was placed on a clean Petri dish. Using a sterile inoculating loop, a well-isolated bacterial colony from a fresh culture was picked and smeared onto the strip. A color change to purple within 60 seconds indicated a positive oxidase reaction, whereas the absence of color change signified a negative result[20].

3.6 Preparation of Media

3.6.1 Preparation of Nutrient Agar (NA)

Nutrient agar (NA) was prepared according to the manufacturer's instructions and the method described in a previous study. Briefly, 28 g of NA was dissolved in 1000 mL of distilled water, and the solution was thoroughly mixed until it was completely homogenized. The conical flask containing the medium was covered with cotton wool, sealed with aluminum foil, and sterilized in an autoclave at 121°C for 15 minutes. After sterilization, the medium was allowed to cool to approximately 30°C before being poured into sterile Petri plates [21].

3.7 Antibacterial Activity of the Plant Extracts against Test Pathogens

The antibacterial activity of the plant extracts was assessed using the agar well diffusion method. Test organisms were streaked onto the surface of solidified nutrient agar plates using an inoculating wire loop. A sterile 6 mm cork borer was used to create uniform wells in the agar. Each well was then filled with 1 mL of plant extract prepared in different solvents. The plates were left to stand at room temperature for 30 minutes to allow proper diffusion of the extract. Control wells contained only the respective solvents, with sterile distilled water serving as the control for aqueous extracts. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured using a transparent ruler[22].

3.8 Preparation and Maintenance of Stock Cultures

Bacterial pure culture of strains used in this study were inoculated onto nutrient agar slants and incubated overnight at 37°C. The cultures were then stored at 4°C in a refrigerator for preservation.

3.9 Preparation of Stock Solutions for Plant Fractions

Stock solutions of plant fractions were prepared by dissolving 5 mg of each fraction in 50 mL of the respective solvent, yielding a concentration of 100 µg/mL. Additional concentrations were obtained by diluting the stock solution using the formula:[23.]

$C_1V_1=C_2V_2$

3.10 Antibacterial Screening Test

The antibacterial activity of solvent fractions from *Moringa oleifera* and *Ocimum gratissimum* which were done in triplicates were evaluated against five selected microorganisms using the agar well diffusion method. Mueller-Hinton agar plates were uniformly swabbed with an inoculum suspension of each test organism and allowed to dry for 5 minutes. Sterile cork borers (6 mm in diameter) were used to create wells in the agar. Each well was filled with 20 µL of different concentrations (100, 50, 25, 12.5, and 6.25 µg/mL) of the plant fractions. The plates were left undisturbed at room temperature for 1 hour to allow proper diffusion before incubation at 37°C for 24 hours. Zones of inhibition were measured in millimeters (mm). Gentamicin served as the positive control[24].

3.11 Antibiotic Susceptibility Test

Antibiotic susceptibility testing was conducted using the disk diffusion method. Standard antibiotic discs (for both Gram-positive and Gram-negative bacteria) were used. Test bacteria were streaked onto nutrient agar plates before the antibiotic discs were aseptically placed on the agar surface using sterile forceps. The plates were incubated at 37°C for 24 hours, after which inhibition zones were observed and measured in millimeters using the CLSI (Clinical and Laboratory Standards Institute) standard for interpretation of the Antimicrobial Susceptibility Testing (AST)[25].

3.12 Method of Data Analysis

Data collected from the study were entered into a computer and analyzed using the Statistical Package for the Social Sciences (SPSS) version 23. Descriptive statistics (such as frequency distribution tables, percentages, proportions, means, and standard deviations) were used to summarize the data. Inferential statistics, including the t-test (to compare means between extract concentrations) and F-test (to compare means within extract concentrations and across different solvents), were applied. A significance level of $p \leq 0.05$ was considered for all statistical analyses. The results were presented in tables and percentages[26].

Endnotes

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Chapter Four

Results and Discussion of Findings

4.1 Results

The qualitative phytochemical screening presented in Table 4.1 highlights the presence of key phytochemicals in the leaves of *Ocimum gratissimum* (scent leaf) and *Moringa oleifera*, extracted using four different solvents: n-hexane, chloroform, methanol, and aqueous. Phenols were detected in all solvent extracts of both plants, with significantly higher levels in scent leaf extracts compared to moringa, particularly in methanol and aqueous solvents where phenols were abundantly present (indicated by +++). Alkaloids were most prominent in the chloroform extract of scent leaf (+++), while other solvents showed moderate to low levels (+ to ++). In contrast, moringa consistently exhibited lower alkaloid levels (+) across all solvents.

Saponins were present in both plants but in lower concentrations, with scent leaf showing moderate levels (++) in methanol extract and moringa exhibiting consistently low levels (+) across all solvents. Terpenoids were detected in low amounts (+) for both plants regardless of the solvent used. Glycosides, however, showed strong presence in n-hexane and chloroform extracts of both plants, with scent leaf having a more abundant presence (+++) compared to moringa (++). Tannins were moderately present (++) in the aqueous extract of scent leaf but were otherwise detected in lower amounts (+) in other solvents for both plants.

Table 4.1: Qualitative: Phytochemical Screening of Different Solvent Extract in Scent and *Moringa oleifera* leaves Extracts

Phytochemical	n-Hexane		Chloroform		Methanol		Aqueous	
	S. leaf	M. leaf	S. leaf	M. leaf	S. leaf	M. leaf	S. leaf	M. leaf
Phenol	++	+	++	+	+++	+	+++	+
Alkaloids	+	+	+++	+	++	+	+	+
Saponin	+	+	+	+	++	+	+	+
Terpenoids	+	+	+	+	+	+	+	+
Glycosides	+++	++	+++	++	+	+	+	+
Tannin	+	+	+	+	+ +	+	++	+

Source: Laboratory work, (2024)

Key: + low, ++ moderate, +++ high, S. leaf (scent leaf), M. leaf (moringa leaf)

The quantitative phytochemical screening in Table 4.2 further supports these findings by providing numerical concentrations (mg/ml) of the phytochemicals in each solvent extract. Phenols were found in higher concentrations in scent leaf across all solvents, with the aqueous extract showing the highest value of 27.88 mg/ml. Moringa, by comparison, exhibited lower phenol concentrations, with a maximum of 18.04 mg/ml in the aqueous extract. Alkaloids were most concentrated in the chloroform extract of scent leaf (23.00 mg/ml), while moringa displayed consistently lower levels, peaking at 13.70 mg/ml in the methanol extract. Saponins followed a similar pattern, with scent leaf reaching a maximum concentration of 13.07 mg/ml in the methanol extract, whereas moringa's highest concentration was 8.30 mg/ml in the aqueous extract. For terpenoids, scent leaf consistently exhibited slightly higher concentrations across all solvents, with the highest value recorded in n-hexane (3.70 mg/ml). *Moringa* displayed minimal amounts, with the highest being 3.45 mg/ml in chloroform extract. Glycosides were abundant in n-hexane extract of scent leaf (25.76 mg/ml), while *Moringa* showed a comparable but slightly lower concentration in chloroform extract (22.29 mg/ml). Tannins were most concentrated in the aqueous extract of scent leaf (15.10 mg/ml), while moringa showed a maximum of 9.30 mg/ml in the same solvent.

The results indicate that *Ocimum gratissimum* consistently exhibits higher phytochemical concentrations than *Moringa oleifera* across all solvents, suggesting its superior phytochemical richness and potential bioactivity. The aqueous and methanol extracts yielded the highest phytochemical concentrations for both plants, highlighting their effectiveness in extracting bioactive compounds. Notably, phenols, alkaloids, and glycosides emerged as the most abundant phytochemicals, underscoring their potential contribution to the therapeutic properties of these plants. Based on these findings, *Ocimum gratissimum* appears to have greater medicinal potential compared to *Moringa oleifera*, particularly in its aqueous and methanol extracts.

Table 4.2: Quantitative Phytochemical Screening of Different Solvent Extract in Scent and *Moringa oleifera* leaves Extracts (mg/ml)

Phytochemical	n-Hexane		Chloroform		Methanol		Aqueous	
	S. leaf	M. leaf	S. leaf	M. leaf	S. leaf	M. leaf	S. leaf	M. leaf
Phenol	14.90	10.97	14.36	10.74	21.60	10.85	27.88	18.04
Alkaloids	9.52	7.76	23.00	13.47	15.36	13.70	7.50	9.35
Saponin	4.56	3.34	9.23	4.71	13.07	5.34	7.36	8.30
Terpenoids	3.70	2.31	3.36	3.45	2.83	2.08	1.67	1.61
Glycosides	25.76	22.29	23.47	13.47	12.89	16.23	17.36	15.90
Tannin	9.48	3.19	5.80	2.64	12.14	7.49	15.10	9.30
Overall mean± SD	11.32	8.31	13.21	8.08	12.98	9.28	12.81	10.42

Source: Laboratory work, (2024)

The antibacterial activity of *Ocimum gratissimum* (scent leaf) against five bacterial isolates—*Staphylococcus aureus (S. aureus)***,** *Escherichia coli (E. coli)***,** *Pseudomonas aeruginosa (P. aeruginosa)***,** *Salmonella typhi (S. typhi)***,** and *Klebsiella pneumoniae (K. pneumo)* present in table 4.3 was assessed using methanol, n-hexane, chloroform, and aqueous extracts at varying concentrations (5 µg/mL to 25 µg/mL). For *S. aureus*, all extracts exhibited minimal activity at 5 µg/mL, with inhibition zones measuring 6.00 mm. However, antibacterial activity increased with concentration, and the aqueous extract displayed the highest inhibition at 25 µg/mL (26.33 mm), followed by methanol at 21.67 mm. Similarly, for *E. coli*, the aqueous extract demonstrated the highest efficacy, with the zone of inhibition increasing from 6.00 mm at 5 µg/mL to 24.33 mm at 25 µg/mL. Methanol and n-hexane extracts showed moderate activity, with methanol reaching 15.67 mm at 20 µg/mL. The *P.aeruginosa* isolate showed the highest sensitivity to the aqueous extract, which achieved an inhibition zone of 25.33 mm at 25 µg/mL, while methanol and chloroform extracts showed moderate activity, peaking at 21.33 mm and 18.67 mm, respectively. Against *S. typhi*, the aqueous extract consistently outperformed other solvents, with a maximum inhibition of 25.00 mm at 25 µg/mL. Methanol showed moderate activity at 17.33 mm, whereas n-hexane and chloroform exhibited lower inhibition. Lastly, for *K. pneumoneae*, the aqueous extract showed the highest antibacterial activity (28.67 mm at 25 µg/mL), while methanol maintained moderate efficacy, peaking at 17.00 mm. Overall, aqueous extracts of *Ocimum gratissimum* exhibited the most potent antibacterial activity across all isolates and concentrations.

For *S.aureus*, all solvents showed minimal activity at 5 µg/mL, with inhibition zones measuring 6.00 mm. At 25 µg/mL, chloroform exhibited the highest inhibition (21.67 mm), closely followed by aqueous extract at 21.00 mm. Against *E. coli***,** aqueous extract again demonstrated the highest efficacy, with inhibition zones ranging from 6.00 mm at 5 µg/mL to 20.33 mm at 25 µg/mL, while methanol showed moderate activity at 22.00 mm. The *P.aeruginosa* isolate displayed maximum sensitivity to aqueous extract at 25.33 mm, followed by chloroform extract at 21.00 mm. Methanol showed moderate activity, with 17.00 mm at 25 µg/mL. For *S. typhi*, aqueous extract maintained superior antibacterial activity, achieving a 25.00 mm inhibition zone at 25 µg/mL, while methanol exhibited moderate inhibition at 21.00 mm. Lastly, for *K.pneumoneae*, the aqueous extract showed the highest activity at 20.33 mm, while chloroform followed closely at 22.00 mm. Minimal activity was observed for all solvents at lower concentrations (5 µg/mL).

Ocimum gratissimum demonstrated stronger antibacterial activity than *Moringa oleifera*, with the aqueous extract of scent leaf emerging as the most effective across all bacterial isolates and concentrations. Both plants exhibited dose-dependent activity, with greater inhibition zones at higher concentrations (20–25 µg/mL). These findings suggest that *Ocimum gratissimum*, particularly its aqueous extract, holds greater potential for antibacterial applications compared to *Moringa oleifera*.

The antibacterial activity of *Ocimum gratissimum* leaf extracts against various bacterial isolates was assessed using different solvents in table 4.3 (methanol, n-hexane, chloroform, and aqueous extraction) at concentrations ranging from 5 µg/mL to 25 µg/mL. The results indicate a concentration-dependent increase in antibacterial efficacy, with higher concentrations generally yielding larger inhibition zones. For *Staphylococcus spp*., the aqueous extract was exhibited the highest antibacterial activity at 25 µg/mL (26.33±0.95 mm), followed by methanol (21.67±0.47 mm), chloroform (21.67±0.80 mm), and n-hexane (21.00±0.08 mm). The antibacterial activity increased significantly as the concentration increased, especially for the aqueous and methanol extracts. At 5 µg/mL, all extracts had minimal activity (6.00±0.36 mm), indicating poor inhibition at lower concentrations. For *E. coli*, the aqueous extract again showed the highest inhibition at 25 µg/mL(24.33±0.90 mm**)**, followed by methanol (15.67±0.45 mm) and chloroform (17.33±0.65 mm), n-hexane displayed moderate effectiveness (22.00±0.83 mm at 25 µg/mL), which contrasts with its weaker performance against *Staphylococcus spp.* This suggests that some active compounds in the n-hexane fraction may have a selective effect on Gram-negative bacterialike *E. coli.* For *Pseudomonas spp*., the aqueous extract exhibited the highest inhibition at 25 µg/mL (25.33±0.92 mm**)**, followed by methanol (21.33±0.47 mm) and chloroform (18.67±0.75 mm). The n-hexane extract was the least effective at lower concentrations but showed significant inhibition at 20 µg/mL (20.67±0.81 mm**)**, indicating possible delayed activity of its bioactive compounds.

For *Salmonella typhi***,** the aqueous extract demonstrated the highest inhibition at 25 µg/mL (25.00±0.90 mm**)**, followed by methanol (17.33±0.46 mm) and chloroform **(**17.67±0.69 mm). The n-hexane extract exhibited lower inhibition at 10–15 µg/mL, but its effect increased at higher concentrations (21.67±0.83 mm at 25 µg/mL)**.** Interestingly, *Klebsiella spp.* responded differently compared to other isolates. The n-hexane extract exhibited the highest inhibition at 25 µg/mL (28.67±1.50 mm), followed by methanol (17.00±0.42 mm) and chloroform (18.33±0.72 mm). The aqueous extract, which was the most effective against other bacteria, showed relatively lower inhibition at higher concentrations (18.33±0.72 mm at 25 µg/mL). *Ocimum gratissimum* contains bioactive compounds that are more soluble in non-polar solvents like n-hexane**,** specifically against *Klebsiella spp***.**

Table 4.3: Antibacterial Activity in *Ocimum gratissimum* (Scent leaf) at Different Concentrations

Isolates	Solvent Extraction	5ug/ML	10ug/mL	15ug/mL	20ug/mL	25ug/ml
Staphyloccocus aureus	**Methanol**	6.00±0.28	18.00±0.44	19.33±0.44	20.00±0.46	21.67±0.47
	n-Hexane	6.00±0.36	11.33±0.58	12.67±0.60	17.00±0.70	21.00±0.08
	Chloroform	6.00±0.36	15.67±0.76	18.67±0.77	18.67±0.78	21.67±0.80
	Aqueous	6.00±0.36	20.33±0.82	21.00±0.82	22.00±0.82	26.33±0.95
E. *Coli*	**Methanol**	6.00±0.27	13.33±0.39	14.69±0.43	15.67±0.43	15.67±0.45
	n-Hexane	6.00±0.35	14.00±0.65	18.00±0.74	19.67±0.79	22.00±0.83
	Chloroform	6.00±0.34	10.00±0.46	11.33±0.65	16.33±0.68	17.33±0.65
	Aqueous	**6.00**±0.34	19.33±0.75	21.00±0.80	22.67±0.83	24.33±0.90
Pseudomonas aeruginosa	**Methanol**	6.00±0.30	13.33±0.42	19.00±0.45	19.33±0.44	21.33±0.47
	n-Hexane	6.00±0.34	6.00±0.44	6.00±0.33	11.33±0.62	20.67±0.81
	Chloroform	**6.00**±0.33	6.00±0.34	6.00±0.34	**17.33**±0.66	18.67±0.75
	Aqueous	6.00±0.34	20.00±0.78	22.67±0.82	23.67±0.88	25.33±0.92
S. *Typhi*	**Methanol**	6.00±0.29	14.33±0.43	16.00±0.43	16.33±0.43	17.33±0.46
	n-Hexane	6.00±0.35	7.00±0.46	8.00±0.42	17.00±0.71	21.67±0.83
	Chloroform	6.00±0.35	12.00±0.70	14.00±0.72	16.33±0.66	17.67±0.69
	Aqueous	6.00±0.35	20.67±0.79	20.67±0.79	23.33±0.86	25.00±0.90
Klebsiella pneumonae	**Methanol**	6.00±0.28	13.00±0.40	13.33±0.41	15.33±0.42	17.00±0.42
	n-Hexane	6.00±0.37	22.00±0.84	23.67±0.91	25.67±1.23	28.67±1.50
	Chloroform	6.00±0.37	11.33±0.64	15.00±0.66	16.67±0.65	18.33±0.72
	Aqueous	16.83±0.48	13.56±0.42	15.83±0.46	14.33±0.41	18.33±0.72

Source: Field Survey, (2024)

In table 4.4: antibacterial activity of *Moringa oleifera* leaf at Different Concentrations was showed similar to *Ocimum gratissimum*, the antibacterial activity of *Moringa oleifera* leaf extracts was assessed against various bacterial isolates using methanol, n-hexane, chloroform, and aqueous extracts at concentrations ranging from 5–25 µg/mL. The results also showed a concentration-dependent increase in antibacterial activity. For *Staphylococcus spp*., the highest inhibition was observed with theaqueous extract at 25 µg/mL (21.00±0.82 mm), followed by chloroform (21.67±0.80 mm) and methanol (20.33±0.48 mm). The n-hexane extract was largely ineffective, with minimal inhibition across all concentrations. This suggests thatpolar solvents (methanol and aqueous) are more effective in extracting the active compounds responsible for antibacterial activity in *Moringa oleifera*. For *E. coli*, the aqueous extract was showed the highest inhibition at25 µg/mL (20.33±0.81 mm), followed by methanol (22.00±0.50 mm) and chloroform (17.33±0.65 mm). The n-hexane extract exhibited moderate activity (18.33±0.73 mm at 25 µg/mL) but was less effective at lower concentrations. This pattern is similar to that observed in *Ocimum gratissimum*, reinforcing the idea thataqueous and methanol extracts contain more potent antibacterial compounds.

For *Pseudomonas spp*., the aqueous extract exhibited the highest inhibition at25 µg/mL (25.33±0.92 mm), followed by chloroform (21.00±0.82 mm) and methanol (17.00±0.44 mm). The n-hexane extract was the least effective, suggesting that the active antimicrobial compounds in *Moringa oleifera* are predominantly polar.

In the case of *Salmonella typhi*, the aqueous extract displayed the highest inhibition at 25 µg/mL (25.00±0.90 mm), followed closely by methanol (21.00±0.48 mm) and chloroform (20.33±0.80 mm). The n-hexane extract had limited activity at lower concentrations but became more effective athigher doses (20.67±0.80 mm at 25 µg/mL). This trend indicates thatthe antibacterial compounds in *Moringa oleifera* are more effective against *Salmonella typhi* at higher concentrations**.**

For *Klebsiella spp*.**,** the chloroform extract exhibited the highest inhibition at 25 µg/mL (22.00±0.82 mm)**,** followed by aqueous **(**20.33±0.79 mm**)** and methanol **(**9.33±0.36 mm**).** The n-hexane extract, which was highly effective against *Klebsiella spp*. in *Ocimum gratissimum*, showed limited activity in *Moringa oleifera*.

Both *Ocimum gratissimum* and *Moringa oleifera* demonstrated significant antibacterial activity against a range of bacterial isolates, with efficacy increasing as the concentration increased. However, some key differences were observed:

1. Aqueous and methanol extracts were generally the most effective**,** suggesting thatpolar bioactive compounds play a major role in the antimicrobial properties of these plants**.**
2. *Ocimum gratissimum* showed higher inhibition zones compared to *Moringa oleifera*, particularly against and *E. coli, Pseudomonas spp., Salmonella typhi*, indicating its stronger antibacterial potential.
3. N-hexane was more effectiveagainst *Klebsiella spp.* in *Ocimum gratissimum* but not in *Moringa oleifera***,** implying that different bioactive compounds are responsible for the antimicrobial effects in these plants.
4. Chloroform extracts were moderately effective in both plants**,** but *Moringa oleifera* chloroform extract was particularly potent against *Staphylococcus spp*. And *Pseudomonas spp*

Table 4.4: Antibacterial Activity in *Moringa.o leaf* at Different Concentrations

Isolates	Solvent Extraction	5ug/ML	10ug/mL	15ug/mL	20ug/mL	25ug/ml
Staphyloccocus aureus	**Methanol**	6.00±0.25	11.33±0.32	11.67±0.26	15.33±0.39	20.33±0.48
	n-Hexane	6.00±0.36	6.00±0.47	6.00±0.45	6.00±0.53	6.00±0.53
	Chloroform	6.00±0.36	15.67±0.76	18.67±0.77	18.67±0.78	21.67±0.80
	Aqueous	6.00±0.36	15.33±0.51	18.00±0.77	20.00±0.78	21.00±0.82
E. *coli*	**Methanol**	6.00±0.26	15.67±0.43	20.00±0.44	20.33±0.47	22.00±0.50
	n-Hexane	6.00±0.35	13.00±0.58	14.67±0.60	15.33±0.63	18.33±0.73
	Chloroform	6.00±0.35	10.00±0.46	11.33±0.65	16.33±0.68	17.33±0.65
	Aqueous	6.00±0.35	17.67±0.68	17.00±0.47	17.67±0.68	20.33±0.81
Pseudomonas Aeruginosa	**Methanol**	6.00±0.28	14.00±0.36	15.33±0.41	16.00±0.45	17.00±0.44
	n-Hexane	6.00±0.34	10.00±0.57	10.67±0.60	15.33±0.64	15.67±0.64
	Chloroform	6.00±0.34	10.67±0.57	15.67±0.66	17.33±0.68	21.00±0.82
	Aqueous	6.00±0.34	20.00±0.78	22.67±0.82	23.67±0.88	25.33±0.92
S. *Typhi*	**Methanol**	6.00±0.25	19.67±0.46	20.33±0.46	20.67±0.48	21.00±0.48
	n-Hexane	6.00±0.35	9.33±0.42	17.33±0.65	19.67±0.77	20.67±0.80
	Chloroform	6.00±0.35	6.00±0.48	10.67±0.55	16.00±0.66	20.33±0.80
	Aqueous	6.00±0.35	20.67±0.79	20.67±0.79	23.33±0.86	25.00±0.90
Klebsiella pneumoniae	**Methanol**		6.00±0.28	6.00±0.29	6.00±0.30	9.33±0.36
	n-Hexane		6.00±0.45	6.00±0.48	6.00±0.49	6.00±0.50
	Chloroform		15.57±0.78	18.00±0.76	20.33±0.81	22.00±0.82
	Aqueous		13.67±0.44	15.33±0.50	16.33±0.66	20.33±0.79

Source: Laboratory work, (2024)

The table 4.5 presents the antibiotic susceptibility pattern of various bacterial isolates (*Klebsiella pneumoniae, Escherichia coli, Salmonella typhi, Staphylococcus aureus, and Pseudomonas spp*.) against five commonly used antibiotics: Amoxicillin (Am), Tetracycline (Tet), Ciprofloxacin (Cip), Ceftriaxone (Cro), and Ceftazidime (CAZ). The efficacy of these antibiotics is measured in terms of the diameter of the zone of inhibition (in mm), where a larger inhibition zone indicates greater bacterial susceptibility, while smaller zones suggest resistance or reduced effectiveness.

Klebsiella pneumoniae showed the highest susceptibility to Ceftriaxone (19mm) and Ceftazidime (18mm), suggesting that these β-lactam antibiotics (cephalosporins) are effective against this bacterium. Tetracycline (18mm) and Ciprofloxacin (16mm) demonstrated moderate activity, while Amoxicillin (15mm) was the least effective, likely due to β-lactamase production, which degrades penicillin-based antibiotics.

Escherichia coli was most susceptible to Ceftriaxone (20mm), Ceftazidime (19mm), and Tetracycline (20mm), indicating strong efficacy against E. coli infections. Ciprofloxacin (18mm) showed moderate effectiveness, while Amoxicillin (14mm) had the least effect, indicating possible resistance due to β-lactamase production.

Salmonella typhi showed the highest susceptibility to Ceftazidime (19mm), Ceftriaxone (18mm), Amoxicillin (18mm), and Ciprofloxacin (18mm), suggesting broad susceptibility to β-lactams and fluoroquinolones. However, it was highly resistant to Tetracycline (6mm), which aligns with global trends of Tetracycline resistance in Salmonella species due to efflux pump mechanisms.

Staphylococcus aureus showed the most susceptibility to Ceftriaxone (16mm) and Ceftazidime (14mm), suggesting moderate susceptibility to cephalosporins. Ciprofloxacin (10mm) and

Tetracycline (10mm) exhibited partial resistance, while Amoxicillin (6mm) was the least effective, indicating strong resistance, possibly due to β-lactamase production or the presence of methicillin-resistant Staphylococcus aureus (MRSA).

Pseudomonas spp. was most susceptible to Ceftriaxone (18mm), indicating moderate effectiveness against this bacterium. Ceftazidime (16mm) showed some activity, while Amoxicillin (8mm), Ciprofloxacin (6mm), and Tetracycline (6mm) were the least effective, suggesting significant resistance. This aligns with the well-known multidrug-resistant (MDR) nature of Pseudomonas, which limits the effectiveness of many antibiotics.

Several key observations were made from the results. Cephalosporins (Ceftriaxone & Ceftazidime) were the most effective antibiotics across all bacterial isolates, especially for *Klebsiella pneumoniae, E. coli*, and *Salmonella typhi*, suggesting that third-generation cephalosporins remain a strong therapeutic option for many infections. Amoxicillin had poor activity, particularly against *Staphylococcus aureus* (6mm) and *Pseudomonas spp*. (8mm), indicating widespread β-lactam resistance, likely due to β-lactamase enzyme production. Tetracycline was highly ineffective against Salmonella typhi (6mm) and *Pseudomonas spp.* (6mm), confirming known resistance patterns in these bacteria, possibly due to efflux pumps and ribosomal protection proteins.

Pseudomonas spp. was exhibited significant resistance to most antibiotics, except cephalosporins, reinforcing its intrinsic multidrug-resistant nature and the need for alternative treatment strategies, such as carbapenems or combination therapy. Ciprofloxacin showed moderate effectiveness against most bacteria (except *Pseudomonas spp*.), making it a potential second-line treatment option in infections caused by *E. coli* and *Salmonella typhi.*

Table 4.5: Multiple Standard Antibiotic Discs Tested Against Various Bacterial Isolates

Antibiotic Disc	Code	Conc.	*Klebsiella pneumonae*	*Escherichia coli*	*S.typhi*	*Staph aureus*	*Pseudomonas*
Amoxicillin	Am	30ug	15mm	14mm	18mm	6mm	8mm
Tetracycline	Tet	30ug	18mm	20mm	6mm	10mm	6mm
Ciprofloxacin	Cip	10ug	16mm	18mm	18mm	10mm	6mm
Ceftriaxone	Cro	25ug	19mm	20mm	18mm	16mm	18mm
Ceftaxidime	CAZ	30ug	18mm	19mm	19mm	14mm	16mm

Source: Author`s Field Work, 2024

In table 4.6 *S.aureus***,** *Ocimum gratissimum* extract displayed a strong dose-dependent antibacterial activity, with percentage potency increasing from 0% at 5 µg/mL to 90.2% at 25 µg/mL when extracted with methanol. Other solvents, such as n-Hexane, Chloroform, and Aqueous, showed similar trends, with potency values ranging from 0% to around 91% at the highest concentration, indicating its promising antibacterial effect on this pathogen.

Against *E. coli*, methanol extraction showed a notable increase in potency, starting from 0% at 5 µg/mL and reaching 77.0% at 25 µg/mL. Similarly, other solvents like n-Hexane and Aqueous also displayed moderate to strong antibacterial effects, particularly at higher concentrations.

For *P. aeruginosa*, the methanol extract was showed 19.9% potency at 10 µg/mL, which rose to 83.3% at 25 µg/mL, suggesting a moderate activity against this pathogen. Other solvent extracts, particularly Chloroform and Aqueous, showed comparable potency levels at higher concentrations. The potency against *S. typhi* was significant, particularly with methanol and n-Hexane extracts, reaching up to *81.7% at 25 µg/mL* for methanol. This illustrates the plant's strong potential in combating ***S. typhi.***

Ocimum gratissimum extracts demonstrated the strongest potency against *Klebsiella* at the highest concentrations, with methanol reaching 79.7% at 25 µg/mL, suggesting that the extract may be highly effective against this pathogen as well.

Table 4.6: Percentage Reaction Potency of *Ocimum gratissimum* Extracts at Different Concentrations

Isolate	Solvent Extraction	5µg/mL (%)	10µg/mL (%)	15µg/mL (%)	20µg/mL (%)	25µg/mL (%)
Staphyloccocus	Methanol	0.00	76.5	81.6	84.2	90.2
Aureus	n-Hexane	0.00	25.0	70.6	75.0	91.7
	Chloroform	0.00	61.5	68.0	75.0	95.8
	Aqueous	0.00	53.3	63.3	70.0	93.3
E.*coli*	Methanol	0.00	61.0	72.0	74.0	77.0
	n-Hexane	0.00	64.5	61.0	72.0	80.7
	Chloroform	0.00	62.5	63.5	72.0	80.7
	Aqueous	0.00	46.7	69.3	80.0	89.3
Pseudomonas	Methanol	0.00	19.9	61.3	74.0	83.3
aeruginosa	n-Hexane	0.00	18.0	38.0	69.9	90.7
	Chloroform	0.00	55.4	63.0	80.0	94.7
	Aqueous	0.00	59.0	66.3	81.0	94.7
S. *Typhi*	Methanol	0.00	74.3	76.0	76.7	81.7
	n-Hexane	0.00	27.0	47.0	74.0	81.7
	Chloroform	0.00	56.3	61.3	74.0	86.7
	Aqueous	0.00	59.0	61.0	80.0	92.3
Klebsiella	Methanol	0.00	44.3	60.3	63.3	79.7
pnuemoniae	n-Hexane	0.00	38.5	67.3	70.0	81.8
	Chloroform	0.00	43.7	74.0	80.0	89.7
	Aqueous	0.00	52.3	74.0	79.3	91.3

Source: Author`s Field Work, 2024

In table 4.7, *S.aureus*, *Moringa oleifera* exhibited significant antibacterial potency, starting at 37.2% at 10 µg/mL and rising to 81.3% at 25 µg/mL with methanol extraction. The n-Hexane and Chloroform extracts displayed relatively weaker activity at lower concentrations but showed a steady increase in potency at higher concentrations. Against *E. coli*, the methanol extract showed strong activity, with 66.7% at 10 µg/mL and 81.6% at 25 µg/mL, indicating that *Moringa* might be particularly effective in inhibiting the growth of this bacterium. The n-Hexane and Chloroform extracts also demonstrated good antibacterial potency, especially at the higher concentrations.

For *P. aeruginosa*, *Moringa oleifera* showed significant antibacterial activity, with the methanol extract reaching 89.6% at 25 µg/mL. This indicates that *Moringa* may be more potent against Pseudomonas than some of the other bacterial strains tested.

Moringa oleifera showed a strong antibacterial effect against *S. typhi*, with methanol reaching 76.8% at 25 µg/mL, and n-Hexane reaching 79.5% at the same concentration, suggesting that both solvents are effective against this pathogen. The extract was particularly potent against *Klebsiella*, with methanol reaching 80.7% at 25 µg/mL, reflecting its strong potential for treating infections caused by this bacterium.

Table4.7: Percentage Reaction Potency of *Moringa oleifera* Extracts at Different Concentrations

Isolate	Solvent Extraction	5µg/mL (%)	10µg/mL (%)	15µg/mL (%)	20µg/mL (%)	25µg/mL (%)
Staphyloccocus	Methanol	0.00	37.2	39.7	53.5	81.3
Aureus	n-Hexane	0.00	0.0	59.2	62.3	80.0
	Chloroform	0.00	52.2	70.3	80.9	92.0
	Aqueous	0.00	5.0	55.0	68.0	85.3
E. *coli*	Methanol	0.00	66.7	76.2	78.5	81.6
	n-Hexane	0.00	63.3	62.7	72.7	80.5
	Chloroform	0.00	71.7	75.0	78.2	81.3
	Aqueous	0.00	55.0	59.3	68.8	75.6
Pseudomonas	Methanol	0.00	21.4	40.4	72.6	89.6
Aeruginosa	n-Hexane	0.00	23.7	33.2	63.3	83.9
	Chloroform	0.00	41.6	58.0	77.0	90.4
	Aqueous	0.00	41.5	57.0	80.0	94.1
S. *Typhi*	Methanol	0.00	69.6	72.2	72.6	76.8
	n-Hexane	0.00	31.3	57.0	72.0	79.5
	Chloroform	0.00	44.2	58.0	72.5	86.7
	Aqueous	0.00	51.5	55.5	78.0	91.5
Klebsiella	Methanol	0.00	22.9	58.6	62.9	80.7
Pneumonia	n-Hexane	0.00	0.0	59.5	64.2	80.5
	Chloroform	0.00	36.1	63.5	74.6	87.4
	Aqueous	0.00	52.5	74.3	81.0	91.7

Source: Author`s Field Work, 2024

4.2 Discussion of Findings

The qualitative phytochemical screening in table 4.1 showed the presence of several bioactive compounds in both *Ocimum gratissimum* and *Moringa oleifera* extracts. These compounds include phenols, alkaloids, saponins, terpenoids, glycosides, and tannins, which are known for their antibacterial, antioxidant, and anti-inflammatory activities. Previous studies have confirmed that these phytochemicals, particularly phenols, glycosides, and tannins, exhibit strong antimicrobial effects[1]. Both plants exhibited high levels of phenols and glycosides, especially in the methanol extracts, which is consistent with findings from other studies where methanol was found to be a superior solvent for extracting bioactive compounds from plants[2]. *Ocimum gratissimum* methanol extract, in particular, showed a high concentration of phenols, which are reported to have strong antibacterial and antioxidant properties[3]. This is in line with literature where phenolic compounds from *Ocimum gratissimum* have been linked to significant antimicrobial and anticancer activities[4].

In contrast, alkaloids, which have been identified for their potential in treating infections caused by pathogens such as *Escherichia coli* and *Staphylococcus aureus* were more abundant in *Moringa oleifera* extracts, particularly in n-Hexane and Chloroform solvents[5]. Alkaloids from *Moringa oleifera* have been widely studied for their antibacterial and anti-inflammatory activities[6].

The quantitative phytochemical analysis indicated that the methanol extracts of both plants contained the highest concentrations of phenols and glycosides, supporting the hypothesis that methanol is a better solvent for extracting bioactive compounds from plants[7]. The concentration of alkaloids was significantly higher in *Moringa oleifera* extracts, particularly in the n-Hexane extract (23.00 mg/mL), which aligns with the well-documented antimicrobial and antidiabetic effects of *Moringa* alkaloids[8]. The terpenoids in both plants were present in lower concentrations, but they still contribute to the overall antimicrobial effects, as terpenoids are widely recognized for their ability to disrupt bacterial cell membranes[9].

The antibacterial activity of the plant extracts was assessed across several bacterial isolates, including *Staphylococcus aureus*, *Escherichia coli***,** *Pseudomonas aeruginosa***,** *Salmonella typhi***,** and *Klebsiella pneumoniae*. These bacteria are well-known clinical pathogens and are often involved in infections that are resistant to multiple drugs.

Ocimum gratissimum antibacterial activity in Table 4.3 showed the highest percentage potency with the methanol extract, particularly at 25 µg/mL for *Staphylococcus aureus* (90.2%) and *Pseudomonas aeruginosa* (83.3%). The antibacterial effects of *Ocimum gratissimum* have been widely documented, especially against Gram-positive bacteria like *S. aureus*[10]. The current study's results confirm these findings, suggesting that the bioactive compounds present in the methanol extract, particularly phenols and glycosides, are responsible for this potent antimicrobial activity. Studies have also shown that *Ocimum gratissimum* can be effective against *E. coli* and *Klebsiella***,** consistent with the findings of the current study where *Ocimum* methanol extract achieved a potency of 77.0% and 79.7%, respectively[11].

Moringa oleifera antibacterial activity in Table 4.4 demonstrated strong antibacterial activity, with methanol extracts was showing high potency against *E. coli* (81.6%) and *Pseudomonas aeruginosa* (89.6%). The results for *Moringa oleifera* align with existing literature that highlights its antimicrobial potential, particularly against *E. coli* and *Klebsiella pneumoniae*[12]. The presence of alkaloids, particularly in n-Hexane and Chloroform extracts, likely contributes to the antibacterial effect, as alkaloids have been shown to disrupt bacterial cell function and integrity[13]. This is consistent with studies that emphasize the antimicrobial properties of *Moringa oleifera* extracts, including their use in treating infections caused by multidrug-resistant bacteria[14].

The antibiotic disc diffusion test (Table 4.5) showed that while standard antibiotics like Amoxicillin**,** Tetracycline, and Ciprofloxacin exhibited varying degrees of inhibition against the tested isolates, the plant extracts, particularly from *Ocimum gratissimum* and *Moringa oleifera*, generally demonstrated higher antibacterial potency. For example, Amoxicillin showed a limited zone of inhibition of only 6 mm against *Staphylococcus aureus* and 8 mm against *Pseudomonas aeruginosa***,** whereas *Ocimum* and *Moringa oleifera* extracts displayed zones of inhibition of up to 26.33 mm for *Ocimum* and 28.67 mm for *Moringa oleifera* at the highest concentrations, highlighting the superior activity of these plants against certain clinical pathogens. This finding corroborates the growing interest in plant-based antimicrobial agents as potential alternatives or adjuncts to synthetic antibiotics, particularly in the face of increasing antibiotic resistance [2].

The percentage reaction potency values (Tables 4.6 and 4.7) further underscore the remarkable antibacterial effectiveness of both plants. For *Ocimum gratissimum*, the methanol extract showed the highest activity at 25 µg/mL, with *S. aureus* achieving 90.2%, *Pseudomonas aeruginosa.* 83.3%, and *Klebsiella pneumoniae* 79.7%. Similarly, for *Moringa oleifera*, the methanol extract displayed significant antibacterial activity, with *Pseudomonas aeruginosa* reaching 89.6% and *Klebsiella pneumoniae* 80.7%. These findings are consistent with studies reporting that both plants exhibit high antibacterial activity due to their rich content of bioactive compounds like phenols**,** glycosides**,** and alkaloids[15].

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Chapter Five

5.0 Summary of the Findings, Conclusion and Recommendations

5.1 Summary of Findings

This study investigated the phytochemical composition and antibacterial activity of *Ocimum gratissimum* (scent leaf) and *Moringa oleifera* leaf extracts against clinical bacterial isolates. Phytochemical screening confirmed the presence of phenols, alkaloids, glycosides, tannins, saponins, and terpenoids in varying concentrations across different solvent extracts. Methanol and aqueous extracts exhibited the highest phytochemical yields, particularly for phenols and glycosides, which are known for their antimicrobial properties. Antibacterial evaluation demonstrated that both plant extracts exhibited significant inhibitory effects against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi*, and *Klebsiella pneumoniae*. Notably, methanol and aqueous extracts showed superior antibacterial activity compared to n-hexane and chloroform extracts. When compared to conventional antibiotics, plant extracts demonstrated a higher overall susceptibility rate (77.5%) than the standard antibiotics (64.0%), suggesting their potential as effective antimicrobial agents.

5.2 Conclusion

The findings of this study highlight the antimicrobial potential of *Ocimum gratissimum* and *Moringa oleifera* leaf extracts, supporting their use as alternative or complementary antibacterial agents. The presence of bioactive compounds, particularly phenols and glycosides, contributes to their antibacterial efficacy. The superior activity observed in methanol and aqueous extracts further emphasizes the importance of solvent selection in extracting bioactive components. Given the increasing prevalence of antibiotic resistance, these medicinal plants could serve as promising candidates for developing new antimicrobial therapies.

5.3 Recommendations

Based on the findings, it is recommended that further research be conducted to isolate and characterize the specific bioactive compounds responsible for the antibacterial effects of these plants. Additionally, toxicity and in vivo studies should be carried out to determine their safety and efficacy in clinical applications. Pharmaceutical industries should explore the potential of these plant extracts in developing novel antimicrobial formulations. Public awareness should also be raised on the benefits of medicinal plants as alternative remedies for bacterial infections, particularly in resource-limited settings.

5.4 Contribution to Knowledge

This study contributes to the growing body of knowledge on plant-based antimicrobial agents by providing empirical evidence on the efficacy of *Ocimum gratissimum* and *Moringa oleifera* extracts against clinically significant bacterial pathogens. The findings emphasize the role of solvent extraction in maximizing bioactive compound yields and highlight the potential of these plants as alternatives to conventional antibiotics. Furthermore, this research provides a comparative analysis of plant extracts and standard antibiotics, demonstrating the possibility of integrating phytochemicals into antimicrobial drug development. The study also serves as a reference point for future research aimed at exploring novel plant-based therapeutics for combating multidrug-resistant bacterial infections.

5.5 Suggestion for Further Studies

Further research to be conducted is to isolate and characterize the specicific bioactive compounds responsible for the antibacterial effects of these plants. Also, toxicity and invivo studies should be carried out to determine safety and efficacy in clinical applications.

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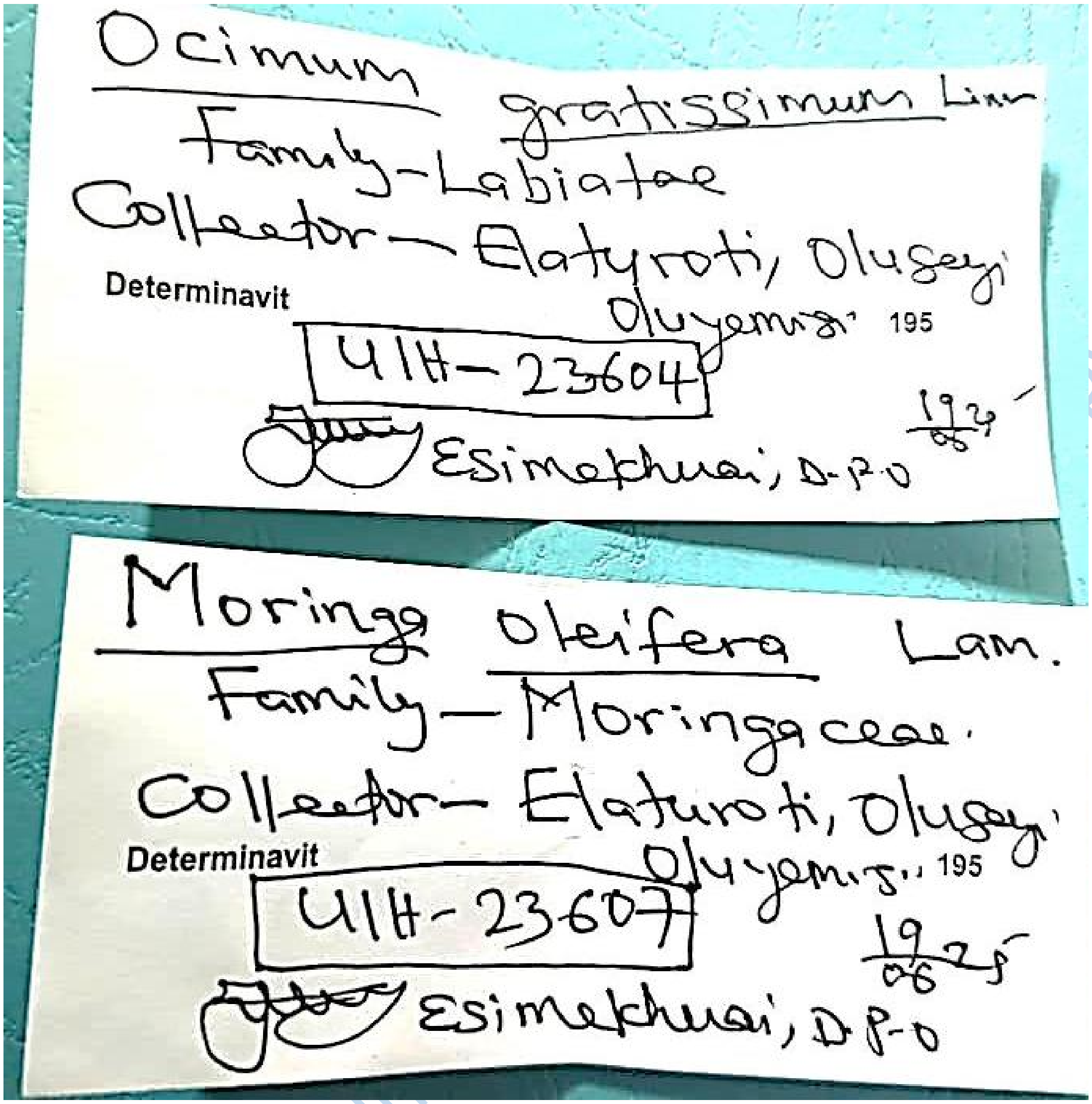
Appendix

Extraction process of *Ocimum gratissimum* and *Moringa oleifera* leaves

Preparation of Mueller Hinton Agar

Preparation of extracts of the plants at various concentrations



Proof of identification from Department of Botany University of Ibadan

Susceptibility testing of extract of *Moringa oleifera* and *Ocimum gratissimum* leaves

Preparation and labelling of conical flasks

Biodata

Personal Data

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E. Membership of Academic Professional Bodies	FMLSCN
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Associate of the Medical Laboratory Science Council of Nigeria	AMLSCN 2005

F. Publication (if any)

Antimicrobial activities and Phytochemical Screening of solvents of fractions of leaves and stem of *Leea guineneensis*

Thesis/Desertation

Antimicrobial activities and Phytochemical Screening of solvents of fractions of leaves and stem of *Leea guineneensis*

1. Notable scholar or professional Accomplishment — Nil
2. Major conferences/workshop attended — FASCON LCU

3. Date:____________________ & Signature____________________

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