PROXIMATE COMPOSITION, AMINO ACID PROFILE, CHEMICAL COMPOSITION, ANTIOXIDANT AND *IN VITRO* ANTI-DIABETIC ACTIVITIES OF *Ocimum gratissimum* STEM BARK EXTRACT

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BENIN CITY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.SC)

OCTOBER, 2025.

CERTIFICATION

This is to certify that this project work was performed by Mirabell Kamsiochukwu Obiefuna with Matriculation number PSC2105227 of the Department of Chemistry, University of Benin, Benin City, Nigeria.

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MIRABELL KAMSIOCHUKWU OBIEFUNA **DATE**

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DR. O.K. OGBEIDE **DATE**
PROJECT SUPERVISOR

_____ _____

PROF. E.E.I. IRABOR **DATE**
HEAD OF DEPARTMENT

DEDICATION

This project research work is dedicated to Almighty God for His wisdom, provision, strength and sound health upon my life throughout this journey.

To my beloved parents, Mr Emeka Obiefuna and Mrs Nneka Obiefuna, for their unwavering support, encouragement and sacrifices.

ACKNOWLEDGEMENT

First and foremost, I give all glory and thanks to the Almighty God for His grace, wisdom, and strength that saw me through the successful completion of this project work.

My sincere appreciation goes to my supervisor, Dr. O.K Ogbeide, for his invaluable guidance, constructive criticisms, and unwavering support throughout this research. I also extend my gratitude to all the lecturers and staff of the Department of Chemistry, Faculty of Physical Sciences, University of Benin, for their academic impact over the years. My appreciation also goes to the Head of the Department, Prof. E.E.I. Irabor, for creating an enabling learning environment. I am equally grateful to the laboratory staff and technologists who assisted me during the experimental procedures of this work. Your support made this work possible. I also express my gratitude to Mr. Okeke David Okechukwu, the owner of Docchy Analytical Laboratory, Awka, Anambra State, for his assistance in the analysis of my bench work.

To my wonderful Parents, Mr. Emeka Obiefuna and Mrs Nneka Obiefuna, for their prayers, financial support, emotional support, encouragement, advice and all their efforts in making my education a success, I say thank you! God bless you both richly.

I also appreciate my project group members; Anita, Ehiosu and Ivie for being good teammates. Special thanks to my friends and classmates, especially Nduoma, Pam Pam, Godswill, Destiny, Chidinma, Success, Chiemelie, Divine, and Itohan, for being a source of help, laughter, peace, motivation and for being my home away from home during my stay on the campus.

To all, I say a heartfelt thank you.

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ABSTRACT

Ocimum gratissimum is a widely recognized medicinal plant known for its diverse therapeutic properties, including antimicrobial, antioxidant, anti-inflammatory, and antidiabetic effects. Its rich content of bioactive compounds has made it a staple in traditional medicine for managing infections, digestive disorders, and general wellness. This study investigates the nutritional composition, amino acid profile, antioxidant potential, enzyme inhibitory activity, and phytochemical constituents of *O. gratissimum* stem extract, providing scientific insight into its medicinal relevance. The qualitative Proximate analysis showed high carbohydrate (53.69%), crude fiber (12.50%), and crude protein (12.26%) content with notable levels of moisture (7.40%), ash (6.27%) and crude fat content (4.92%). Amino acid profiling revealed abundant essential and non-essential amino acids, notably Isoleucine (29.42 mg/ml) and Alanine (22.55 mg/ml). *In vitro* Total Antioxidant Capacity assay demonstrated significant antioxidant activity, with ascorbic acid values increasing from 0.31 to 0.52 mg/ml as the concentration rose from 0.1 to 0.3 mg/ml, while the *In vitro* Nitric Oxide Scavenging Assay showed a progressive increase in the %NO Scavenging from 42.48% to 55.03% for concentrations of 0.1 to 0.3 mg/ml, confirming significant radical scavenging ability. The extract also showed strong alpha amylase (6**0**.43%) and alpha glucosidase (76.91%) inhibition. GCMS identified 35 bioactive compounds, with Erucic acid (with a peak area of 4.59%), D-carvone (5.78%), Oleic Acid (8.90%), Cyclopropane (17.01%) and 9 borabicyclo compounds (28.30%), having the most prominent peak areas. These results suggest the extracts potential in nutritional and therapeutic applications.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Medicinal plants are a valuable source of a wide variety of chemical molecules having different functionalities and structures that exhibit important biological activities and are tied to a multitude of beneficial properties, such as antioxidant and enzyme inhibitory, antimicrobial, anticancer, antiviral, anti-aging, anti-inflammatory, antihypertensive, neuroprotective and anticoagulant effects (Ali *et al*., 2019; Lesellier *et al*., 2021). Throughout the ages, natural products have played a significant role in health care and prevention of diseases. Humans have relied on nature to cater for their basic needs; food, shelter and medicines for the treatment of a wide spectrum of diseases (Osayemwenre *et al.,* 2025). Healing with Medicinal plants is as old as mankind itself. The use of natural bioactive compounds from medicinal plants in the treatment of diseases has been dated back to man's origin. So many infections and diseases have been treated using herbal medicine by herbalists and traditionalists without the full knowledge of the major ingredients in the plant that brought about the cure of the disease (Oscar and Ogbeide 2022). The connection between man and his search for drugs in nature dates from the far past, of which there is ample evidence from various sources: preserved monuments, written documents and even original pony medicines (Petrovska 2012). Hence, plants have been employed as antioxidants, anti-diabetic, anti-inflammatory, anti- carcinogenic, antimicrobial and antioxidant drugs (Ogbeide *et al*., 2022)

Plants synthesize hundreds of chemical compounds for various functions, including defense and protection against insects, fungi, diseases, parasites (Francois *et al.,* 1999) and herbivorous mammals (Gershenzon *e*t Ullah, 2022) and these chemicals are called phytochemicals. Medicinal plants are widely used as folk medicine in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines. Due to their excessive pharmacological ability, plants continue to be used in traditional drugs to treat different ailments (Ogbeide *et a*l., 2018). Several

wholesome products are formed from plants and have remained in use as long established remedies for various human illnesses for many decades (Lichterman 2004, Iyasele *et al.,* 2022).

1.1.1. BACKGROUND OF STUDY

Human beings have depended on nature for their needs, as nature has become a fundamental source for medicines, foodstuffs, shelters, fragrances, clothing, flavors, fertilizers and means of transportation throughout the ages (Roopa Kulkarney 2023).
Medicinal plants are a valuable source of a wide variety of chemical molecules having different structures and functionalities that exhibit important biological activities and are linked to a multitude of beneficial properties, such as anti-cancer, anti-viral, anti-microbial, antioxidant and enzyme inhibitory, anti-aging, anti-inflammatory, anti-hypertensive, neuroprotective and anti-coagulant effects (Ali *et al*., 2019, Lesellier *et al.,* 2021).

Nigeria is abundantly endowed with native plants which are used in herbal medication to remedy ailments and heal injuries. Some of these plants are used as medicine or food. The minerals, vitamins and phytochemicals present in these plants are extensively believed to be the therapeutic constituents that actively contribute to these protective benefits (Okwu and Ekeke 2003, Okwu 2004).

Ocimum gratissimum belongs to the group of plants known as spices. *Ocimum gratissimum* (labiatae) generally known as scent leaf is widely distributed in tropical and warm temperate regions. Considering the fact that *Ocimum gratissimum* is used in most local dishes to achieve a variety of purposes, there is need to ascertain if it's extract antagonizes or acts as a synergy when used together with conventional antibiotics (Nweze *et al*., 2009).

1.1.2. STATEMENT OF PROBLEM

Despite the increasing global interest in natural remedies and medicinal plants, the full therapeutic potential of many widely used plants like *Ocimum gratissimum* remains under-researched or poorly documented in scientific literature. While it is traditionally used in various cultures for treating a range of ailments such as infections, inflammation and diabetes, there is a need for scientific validation of these claims through phytochemical screening, elemental analysis and biological assays. These claims mostly have no scientific basis but are generally based on testimonies and other anecdotal reports from individuals who have used such remedies. The scientific community thus needs much more proof to know the truth about the characteristics, safety and efficacy of such remedies and possible complications from their use (Joseph *et al*., 2021).

1.1.3. JUSTIFICATION/RELEVANCE OF STUDY

Recent trends in global healthcare reveal mounting interest in plant-based therapies. These natural alternatives are increasingly perceived as safer, more affordable options compared to synthetic pharmaceuticals that often carry potential adverse effects. Research focusing on *Ocimum gratissimum* appears particularly promising (Bandaranayake *et al.,* 2006).

Ocimum gratissimum (scent leaf) is widely known for its culinary and traditional medicinal uses, yet the scientific exploration of its stem bark remains limited compared to its leaves. Investigating the proximate composition, amino acid profile, chemical constituents (via GC-MS), as well as its antioxidant and in vitro anti-diabetic activities, offers valuable insight into its nutritional and therapeutic potentials. This study is justified by the increasing global interest in plant-based solutions for oxidative stress and diabetes management, and the need to validate the pharmacological claims surrounding this plant through evidence-based research. Understanding its biochemical composition can contribute to the development of natural health products and functional foods.

1.1.4. SCOPE OF THE WORK

This research work focuses on the following:

1. Proximate Composition
2. Amino Acid Profile
3. Chemical Constituents Identification via GCMS
4. *In vitro* Antioxidant Activity
5. *In vitro* Anti-diabetic studies

1.1.5. AIM AND OBJECTIVES

1.1.5.1. AIM

To determine the proximate composition, amino acid profile identify bioactive compounds and chemical constituents via GC-MS analysis, and investigate the *In-vitro* antioxidant and *In-vitro* anti-diabetic activities of the extract of *Ocimum gratissimum* stem.

1.1.5.2. SPECIFIC OBJECTIVES

To attain the aim, the subsequent objectives are stated;

- to collect, dry and grind the stems of *O.gratissimum*
- to extract the ground stem using ethyl acetate
- to evaluate the proximate composition of the stem extract of *O.gratissimum*
- to determine the amino acids present in the stem extract
- to identify the chemical constituents present in the stem extract of *O.gratissimum*
- to determine the antioxidant activity of the stem extract of *O. gratissimum*
- to ascertain the anti-diabetic action of the stem extract.

1.2. MEDICINAL PLANTS

Medicinal plants, also known as herbal drugs, are defined as botanical raw materials that are primarily utilized for therapeutic, aromatic and culinary purposes in cosmetics, medicinal products, health foods and other natural health products (Jean *et al.,* 2013).
A valuable source of a wide variety of chemical molecules having different structures and functionalities that exhibit important biological activities and are linked to a multitude of beneficial properties such as anti-microbial, anti-cancer, antiviral, antioxidant and enzyme inhibitory, anti-aging, anti-inflammatory, anti-hypertensive, neuroprotective and anti-coagulant effects are medicinal plants (Ali *et al*., 2019; Lesellier *et al.,* 2021).

A remarkable number of reports on the therapeutic properties of medicinal plants combined with long term experience in folk medicine has led to a growing interest in the use of natural products (Tlili and Sankurkcu, 2020). According to various sources, 25-50% of currently produced drugs used in health care are gotten from medicinal plants (Mahmood *et al.,* 2019; Sinan *et al.,* 2020), and new bioactive compounds from known and exotic plants are being sought around the world (Fettach *et al*., 2019).
The presence of substantial biological and pharmacological activities like antioxidant, anti-diabetic, anti-inflammatory, anti-microbial, anti-cancer, hepatoprotective, anti-hypertensive, anti-diarrheal and anti-mutagenic characteristics in *Ocimum gratissimum* have been demonstrated by many pharmacological studies.

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1.3 LITERATURE REVIEW

Ocimum gratissimum, popularly known as “Scent Leaf”, “Clove Basil” or “African Basil”, is one of the discovered medicinal plants with the potential to serve as an alternative therapy for the treatment of various ailments or as a source of a new drug (Ositadinma *et al.,* 2021). It belongs to the family **Lamiaceae** and is found in Africa, Asia and South America (Tanko *et al.,* 2008; Akara *et al*., 2021). Plants of the Lamiaceae family are mostly classified as spices, herbs and other aromatic variations. The genus *Ocimum* comprises about 60 species, of which most are found in Africa (Tank *et al.,* 2008). *O.gratissimum* is a perennial and odoriferous shrub found in tropical regions such as Brazil, India, Vietnam, Rwanda, Nigeria (Lahlou *et al.,* 2004; Nweze

and Eze 2009), Cameroon, Togo, Côte d'Ivoire, Kenya, Benin (Kpoviessi *et al.,* 2014) and South Africa (Venuprasad *et al.,* 2014). Its medicinal potential in Africa is incredibly vast and varies by Country (Kpoviessi *et al.,* 2014).

It is about 1-3 m tall (depending on environmental conditions such as soil, water availability and sunlight), has an erect stem, and is branched, round-quadrangular, and woody at the base. The leaves are opposite, ovate-lanceolate, and have a strong clove-like aroma due to its high essential oil content, particularly Eugenol. The plant bears small white or purplish flowers arranged in spikes and is propagated through seeds or stem cuttings.

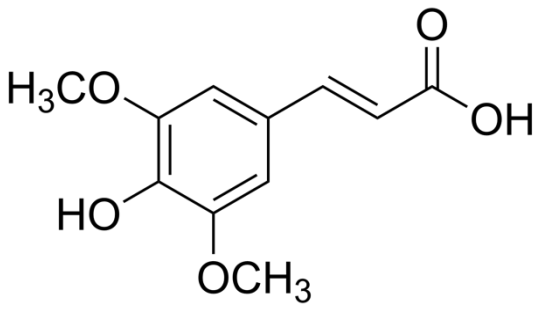
Traditional Names for the plant include: "***Efirin***"- in Yoruba, "***Nchanwu***"- in Igbo, "***Daidoya***"- in Hausa, "***Ebe-amwonkho***"- in Edo, "***Añyeba***"- in Igala and "***Ntong***"- in Efik. In Nigeria, it is used in making pepper soup, local rice, beans, plantain and many other delicacies. The seeds of *O. gratissimum* tend to need strong sunlight to germinate although germination has been seen to have been achieved even during an average UK summer (Martins *et al.,* 2008). *O. gratissimum* repels the thrips "*Thrips tabaci"* and so is a useful insect repellent in other crops (Kirk *et al.,* 2021). Scientific reports have shown that *O. gratissimum* has a wide range of bio active compounds such as flavonoids and poly phenols (Venuprasad *et al.,* 2014; Irondi *et al*., 2016) and essential oils with several beneficial effects (Benitez *et al 2009;* Menlo *et al.,* 2019).

O. gratissimum is used in the treatment of a variety of condo such as; aches, fever, inflammation, diarrhea, anaemia, fungal and bacterial infections (Shedoeva *et al.,* 2019). The essential oils of *O. gratissimum* leaf contains significant amounts of eugenol, camphor, limonene, thymol, pinene and other chemical compounds that are responsible for many of its medicinal properties (Akpoghelie *et al.,* 2022).

O. gratissimum possesses calcium and magnesium, both of which work in lowering blood sugar (Okoduwa *et al.,* 2017). It also aids the heart to function properly by removing toxic substances and cholesterol which the body does not need (Ademiluyi *et al*., 2023).

The Chemical structures and biological activities of the compounds isolated from *O. gratissimum* are highlighted below:

1. **Sinapic Acid**



Sinapic Acid

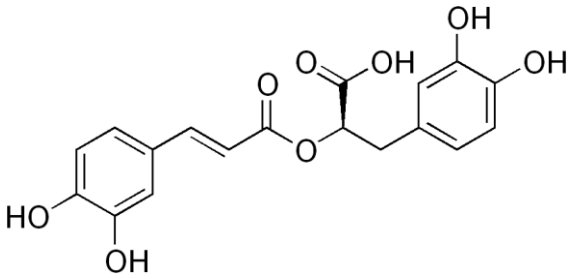
Molecular Formula: $C_{11}H_{12}O_5$

Molecular weight: 224.21g/mol

IUPAC Name: (2E)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid

It exhibits antioxidant, anti-inflammatory, anti cancer, anti mutagenic, anti glycemic, neuroprotective and antibacterial activities (Chen 2016).

2. **Rosmarinic Acid**



Rosmarinic Acid

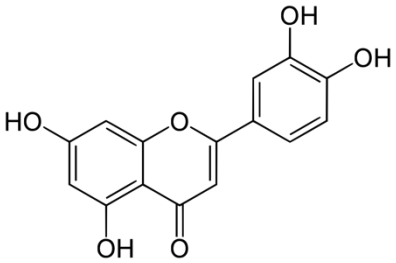
Molecular Formula: $C_{18}H_{16}O_8$

Molecular Weight: 360.32g/mol

IUPAC Name: (2R)-3-(3,4-dihydroxyphenyl)-2-{[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}propanoic acid.

It exhibits anti-microbial, immunomodulatory, anti-diabetic, anti-allergic, anti-inflammatory, hepato and renal-protectant agent. (Alagawany *et al.,* 2017).

3. **Luteolin**



Luteolin

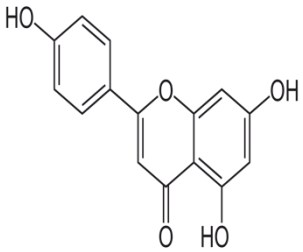
Molecular Formula: $C_{15}H_{10}O_6$

Molecular weight: 286.24g/mol

IUPAC Name: 2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one

It exhibits anti hypertension, anti inflammatory and anti-cancer activities (Lin *et al., 2008).*

4. **Apigenin:**



Apigenin

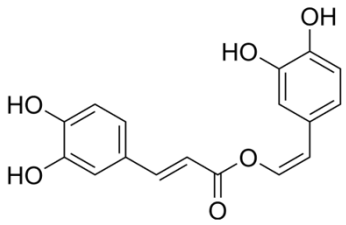
Molecular Formula: $C_{15}H_{10}O_5$

Molecular Weight: 270.24g/mol

IUPAC Name: 5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one

It exhibits anti-inflammatory, antioxidant, antibacterial, antiviral activities and blood pressure reduction (Yan *et Al.,* 2017).

5. **Nepetoidin:**



Nepetoidin

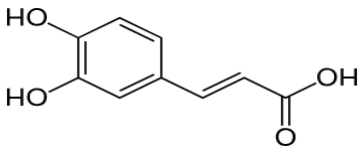
Molecular Formula: $C_{17}H_{14}O_6$

Molecular Weight: 314.29g/mol

IUPAC Name: [(Z)-2-(3,4-dihydroxyphenyl)ethenyl] (E)-3-(3,4-dihydroxyphenylprop-2-enoate

It exhibits antioxidant, antiviral, anti fungal, antibacterial effects, Xanthrine oxidase, nitric oxide inhibitor (Grayer *et al.,* 2003, Tsai and Lee 2014).

6. **Caffeic Acid:**



Caffeic Acid

Molecular Formula: $C_9H_8O_4$

Molecular Weight: 180.16g/mol

IUPAC Name: (2E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid.

It exhibits antioxidant, anti-inflammatory, anti carcinogenic activities. (Ye *et al.,* 2010, Espíndola *et al., 2019).*

1.4 TAXONOMY AND CLASSIFICATION OF OCIMUM GRATISSIMUM

Fig 1: Ocimum gratissimum

Common Names: "***Efirin***"- in Yoruba, "***Nchanwu***"- in Igbo, "***Daidoya***"- in Hausa, "***Ebe-amwonkho***"- in Edo, "***Añyeba***"- in Igala and "***Ntong***"- in Efik.

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Lamiales

Family: Lamiaceae

Genus: Ocimum

Species: gratissimum

Height: 1-3 meters

Type: Herb

Native Range: Tropical Africa, India and South-east Asia

Width: 0.5 – 1 meters

The leaves of *O. gratissimum* are simple, opposite, dentate and carried by a pubescent petiole of 2 to 5cm long. The blade is elliptical to oval, 1.5 – 2.5cm long and 0.6 to 1.2cm wide. The margin is coarsest serrated. The apex and vase are pointed. Both sides are covered with short dense hairs. Venation is pinnate, arched.

The stem is rounded-quadrangular, highly branches, woody, rigid and has its epidermis peeling off in strips at the base, more or less glabrous but becoming pubescent at nodes and on the inflorescence axis. The flowers have several colors such as yellowing white, greenish purple, hairy calyx, brown seeds and they are not slimy (Chowdhury *et al 2017).*

The fruits are formed of spherical capsules of about 2mm in diameter. It has a tap root system. In Africa, Eastern, Central and Western Kenya, *Ocimum gratissimum* is commonly found in scrub and disturbed highland forests at elevations of 600-2400m above sea level (Ogendo *et al., 2008).*

1.5 EXTRACTION

Secondary metabolites of plants are of major interest for pharmaceutical, cosmetic and food applications. The extraction methods for these compounds must be optimized to achieve the best possible yield without altering the effectiveness of the targeted compounds (Reda and Ahmed 2023).

Extraction methods for natural plant-derived compounds are essential processes used to isolate bio active molecules, flavors, fragrances and other valuable substances from plants (El Boukhari and Fatimi 2023). However it is essential to note that these methods vary depending on the type of compounds, plant source and equipment used (Abubakar and Haque 2020).

Extraction methods include **solvent extraction, distillation method, pressing** and **sublimation** according to the extraction principle (Zhang *et al* 2018). Among these,

Solvent extraction is the most commonly used method. Some important terms in the Solvent extraction process include:

- **Menstruum:** This is a liquid or suitable solvent chosen for an effective extraction process (Azwanida 2015; Pandey and Tripathi 2014).
- **Marc:** This is an insoluble or inert drug material that is left behind at the end of the extraction process.
- **Micelle:** It is the mixture of both the extracted drug material and the solvent of extraction.

Extraction remains the primary and most crucial phase in the investigation of medicinal plants since it is essential to distinguish and identify the necessary chemical constituents inherent in plant materials and its effectiveness strongly increases the quality of the final results.

The conventional extraction methods including **maceration**, **percolation, decoction and reflux extraction** usually use organic solvents and require a large volume of solvents and long extraction time (Zhang *et al* 2018).

- **Maceration:** This is a very simple extraction method that involves several steps, namely; grinding, addition of suitable solvent and straining off the liquid. The pressed out liquid and strained mass are mixed and separated by means of filteration (Majid *et al.,* 2023). It can be used for the extraction of thermolabile products.
- **Percolation:** This is more efficient than maceration because it is a continuous process in which the saturated solvent is constantly being replaced by fresh solvent. (Zhang *et al.,* 2018).
- **Decoction:** The extract from decoction contains a large amount of water-soluble impurities. It cannot be used for the extraction of thermolabile or volatile components (Zhang *et al.,* 2018).
- **Reflux Extraction:** This is more efficient than percolation or maceration and requires less extraction time and solvent. It can't be used for the extraction of thermolabile products. It is a solid-liquid extraction technique where a volatile solvent is repeatedly evaporated, condensed and returned to the extraction vessel

at a constant temperature for a set period to efficiently extract the compounds (Zhang *et al.,* 2023).

Solvent extraction may be the most popular, but there are many other methods ranging from older to newer and from simple to more complex. Choosing the right extraction method for a given ingredient or product requires consideration of the characteristics of each available method.

The choice of an appropriate extraction method depends on the nature of the plant material, solvent used, pH of the solvent, temperature and solvent to sample ratio. It also depends on the intended use of the final product (Ingle *et al.,* 2017, Azwanida *et al.,* 2015).

1.6 PROXIMATE ANALYSIS

Proximate Analysis arises from the term "*approximate analysis"* indicating that they are not exact/accurate (Eija Alakangas 2016). It plays a significant role in the food industry by providing critical insights into the nutritional value of food products.

Proximate Analysis is the partitioning of compounds in a feed into six categories based on the chemical properties of the compounds. The 6 categories are:

- Moisture content
- Ash content
- Crude Protein content
- Crude Fat content
- Crude Fiber content
- Carbohydrate content

1.6.1 MOISTURE CONTENT

This refers to the overall weight of water/moisture contained in a plant/food material. It is usually expressed as a percentage of weight (Jerome Vanclay 2009). It is a very important parameter in the analysis of food, pharmaceuticals and plants. There are numerous reasons why moisture content analysis is very critical in the proximate analysis of a material. Some of which are:

- High moisture content levels promote the growth of microorganisms thereby increasing the rate of spoilage.
- Moisture content helps determine the appropriate drying requirements a particular material needs to avoid spoilage.
- Consistent moisture content is essential for the standardization of plant-based products.
- Many bioactuve compounds degrade in the presence of excess water therefore it is controlled to preserve the antioxidant, anti inflammatory and anti microbial properties of plants.

1.6.2 ASH CONTENT

Ash content refers to the minerals and inorganic substances left after heating a particular plant sample at extremely high temperatures to remove all organic materials (Precisa 2025). These inorganic substances are oxidized during the combustion process and take the ash form (Murat *et al* 2020). This ash residue includes essential and non-essential minerals such as calcium, magnesium, potassium, sodium, phosphorus, iron etc. Ash content analysis helps to evaluate the quality and purity of plants for herbal formulations. It also aids in determining the presence of impurities in the sample such as sand or soil.

1.6.3 CRUDE PROTEIN

Crude Protein analysis determines the total protein content in a sample by first measuring the amount of nitrogen present in the sample and multiplying by a conversion factor to estimate the total crude protein. The conversion factor usually applies is 6.25. This is because, via the basis of early determinations, the average nitrogen content of proteins was found to be about 16%, making the conversion factor: **100/16 = 6.25.**
While 6.25 is the standard for general purposes, specific factors may be used for different food types. The two most widely used techniques for total determination of nitrogen content are the Kjeldahl method (Kjeldahl J.Z 1883) and the Dumas method (Dumas *et al.,* 1831).

The Kjeldahl method entails the digestion of a sample and the subsequent quantification of its nitrogen content (Sarmila KC 2023).

1.6.4 CRUDE FAT

Crude Fat is a term used to refer to the crude mixture of fat-soluble materials present in foods, also known as ether extract or free lipid content. It is the total amount of extractable lipids in plant materials using non-polar solvents like ether or hexane. These lipids in the plant material include triglycerides, phospholipids, sterols, fatty acids, fat soluble vitamins etc.

1.6.5 CRUDE FIBER

This refers to the insoluble dietary fiber fractions. Crude fiber is the organic material left over when plant materials are dissolved in a weak solution of an acid and later in a solution of a base. Crude fiber comprises the constituents of the cell walls found in plant tissues, including cellulose, hemicellulose and lignin (McDonald and Whitesides 2002).

1.6.6 CARBOHYDRATES

A carbohydrate is a biomolecule composed of C, H and O atoms. The term “total available carbohydrate” may be defined as all those carbohydrates which can be used in the plant as a source of energy or building material, either directly or indirectly (Weinmann.H. 1943). The carbohydrate content is not typically measured directly but is calculated after determining other components like moisture, ash, protein, fat and fiber.

1.7 AMINO ACIDS

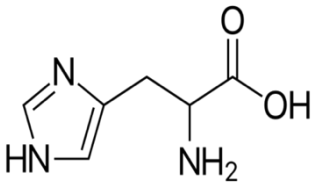
Amino Acids are organic compounds that contain both amino and carboxylic acid functional groups (Nelson and Cox 2005) and an organic R-group that is unique to each amino acid (Reddy 2025). The R-group represents a side chain specific to each amino acid, and there are 20 different kinds of side chains that are commonly found in proteins (Milne and Kilian 2010).

Amino Acids function as the building blocks of proteins. In the human body, there are 20 amino acids that function as building blocks of proteins. Out of these 20, they are classified into essential and non-essential amino acids.

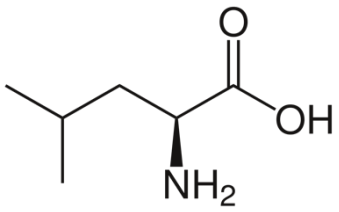
1.7.1 ESSENTIAL AMINO ACIDS

These are the amino acids that the body cannot synthesize on its own. Therefore they must be supplied from an exogenous diet (Michael and Shamin 2024). They are vital for protein synthesis, tissue repair and overall bodily functions. There are 9 essential Amino acids:

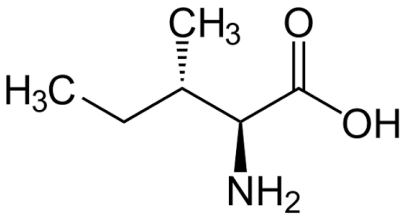
- Histidine



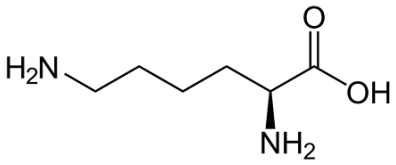
- Leucine



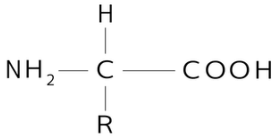
- Isoleucine



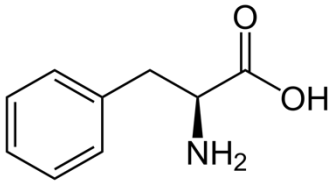
- Lysine



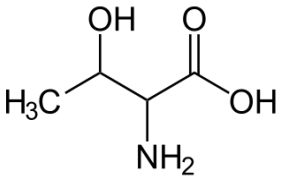
- Methionine



- Phenylalanine

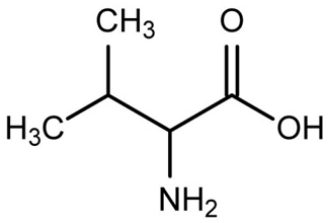


- Threonine



- Tryptophan

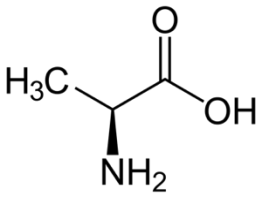
- Valine



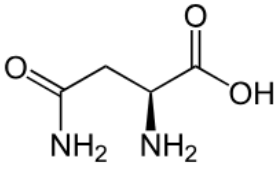
1.7.2 NON-ESSENTIAL AMINO ACIDS

These are the amino acids that the human body is able to synthesize on its own. This means they do not need to be ingested through an external diet. Cells in the body can provide the carbon skeleton of the non-essential amino acids (Gerald 2018). Although they are said to be non-essential, it however does not make them unimportant. They aid in the removal of toxins, are integral in the synthesis of red and white blood cells and they promote brain function. There are 11 non-essential amino acids:

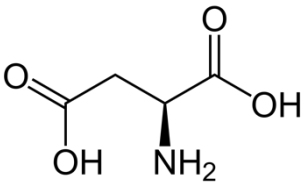
- Alanine



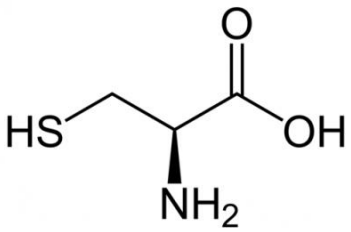
- Asparagine



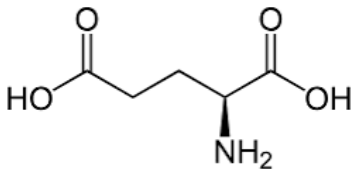
- Aspartic acid



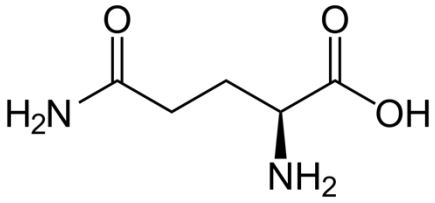
- Cysteine



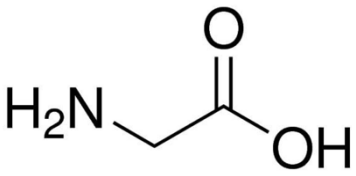
- Glutamic acid



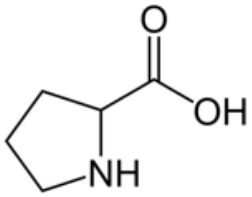
- Glutamine



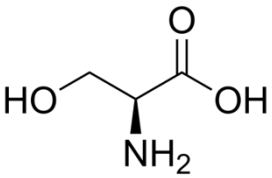
- Glycine



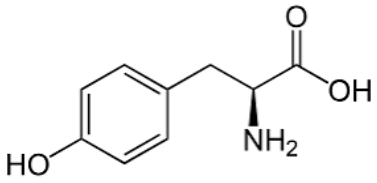
- Proline



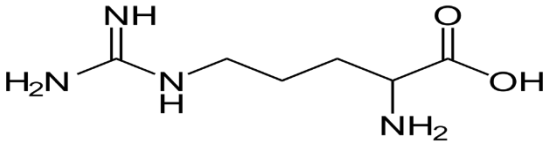
- Serine



- Tyrosine



- Arginine



1.8 ANTIOXIDANTS

When oxygen is metabolized, it creates unstable molecules called “free radicals” which steal electrons from other molecules causing damage to DNA and other cells. Overtime, the damage caused by an overload of free radicals may become irreversible and lead to certain diseases and even cancer. Antioxidants are a group of compounds that neutralize free radicals and Reactive Oxygen Species (ROS) in the cell (Abuajah *et al.,* 2015). They turn the neutralized free radicals into unwanted byproducts which are then removed from the body. Antioxidants can be classified into two basic groups: **Natural Antioxidants** and **Artificial Antioxidants**. Natural antioxidants are substances that exist in foods and prevent their reactions such as disruption, sourness and colour change. They are generally derived from plant sources and their activity varies depending on plant species, diversity, extraction methods and growing conditions. They are found in microorganisms, some animal tissues and almost all plants (Zehirohlu and Ozturk 2019).

The various methods for evaluation of the antioxidant capacity fall into three distinct categories namely, spectrometry, electrochemical assays and chromatography

(Moharram and Youssef 2014). Some of the most common antioxidant assays are as follows:

- Ferric Reducing Antioxidant Power: FRAP, or Ferric Reducing Antioxidant Power assay, is defined as a method that measures the antioxidant capacity of a sample by assessing its ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in a redox reaction, resulting in a color change that is quantitatively analyzed spectrophotometrically. The degree of color change is directly proportional to the antioxidant activity present in the sample (Khursheed et al., 2024). The formula for the FRAP assay involves comparing the absorbance of your sample to a standard curve of known antioxidant (like ascorbic acid), not a single fixed equation.

- Nitric Oxide Scavenging Assay: The nitric oxide (NO) scavenging assay measures a substance's ability to neutralize excess nitric oxide, a free radical that contributes to inflammatory processes. The principle relies on NO, often generated from a stable precursor like sodium nitroprusside, reacting with an added test compound. This reaction is typically quantified using the Griess reaction, which detects the decrease in NO or its subsequent conversion product (nitrite) in the presence of the test compound compared to a control. The NO scavenging assay formula to calculate percent inhibition is:

  $$\textbf{\%NO Scavenging:}[(A_0 - A_1) / A_0] \times 100,$$

  where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. This formula quantifies how much of the nitric oxide (NO) radical is "scavenged" or removed by a test compound compared to a control.
- DPPH Radical Scavenging Assay; DPPH radical scavenging activity is a widely used laboratory method to measure the capacity of antioxidant compounds to neutralize free radicals, which are unstable molecules that can damage cells. The assay works by reacting antioxidants with a stable, purple-colored free radical called DPPH (2,2-diphenyl-1-picrylhydrazyl); when the antioxidant "scavenges" (neutralizes) the radical, the purple color disappears, changing to a yellow hue, which is measured spectrophotometrically to determine antioxidant capacity.

The equation for DPPH radical scavenging activity (RSA) is typically expressed as a percentage of inhibition:

% RSA = [(Absorbance of control - Absorbance of sample) / Absorbance of control] × 100.

In this formula, the "control absorbance" is the absorbance of the DPPH solution without the antioxidant sample, and the "sample absorbance" is the absorbance of the DPPH solution with the added antioxidant. A higher percentage indicates a greater ability to scavenge the DPPH radical.

Several *in vivo studies* carried out in the 80s and 90s reported some health risks associated with the consumption of synthetic antioxidants (Mut-Salud *et al.,* 2016). Findings in recent years have also shown that synthetic antioxidants may exhibit toxic effects, require high cost and show less efficacy than natural antioxidants (Zehiroglu and Ozturk 2019). For this reason, interest in natural antioxidant consumption has increased drastically.

The body is capable of producing some antioxidants on its own, and these antioxidants are referred to as **Endogenous Antioxidants.** A good example is glutathione.

On the other hand, antioxidants that can only come from outside the body are called **Exogenous Antioxidants.** They are gotten from fruits, vegetables and other plant based Whole Foods

Adequate antioxidant intake is very essential. One's life literally depends on the intake of certain antioxidants. Namely; Vitamins C and E. Berries, coffee, green tea and dark chocolate are good sources of antioxidants (Carlson *et al.,* 2010).

1.8.1 USES OF ANTIOXIDANTS

- Antioxidants are used to prevent oxidation which is a form of chemical degradation.
- Antioxidants can increase the shelf life of both natural and processed foods.
- Antioxidants are added to industrial products such as stabilizers in fuels and additives in lubricants to prevent oxidation and polymerization that leads to the formation of engine fouling residues (Klemchuk Peter 2000).

1.9 ANTI-DIABETIC STUDIES

1.9.1 DIABETES

Diabetes mellitus, commonly known as diabetes, is a group of common endocrine diseases characterized by sustained levels of high blood sugar (WHO 2023). Diabetes occurs either due to the pancreas not producing enough of the insulin hormone or the cells of the body becoming unresponsive to the effects of the insulin (Shoback and Gardner 2011). Insulin is a hormone that regulates blood glucose. In 2022, 14% of adults aged 18 years and older were living with diabetes, an increase from 7% in 1990. More than half (59%) of adults aged 30 years and over living with diabetes were not taking medication for their diabetes in 2022.

Classic symptoms of diabetes include: excessive thirst (polydipsia), excessive urination (polyuria), excessive hunger (polyphagia), weight loss and blurred vision. However if treatment is delayed or denied, the disease can lead to various and more several health conditions, including disorders of the cardiovascular system, eye, kidney and nerves (Kitabchi *et al.,* 2009). Diabetes is responsible for approximately 4.2 million fatalities every year (IDF DIABETES ATLAS 2020).

1.9.2 TYPES OF DIABETES

There are two major types of diabetes. They are:

- **Type 1 Diabetes**: This is an autoimmune condition where the body's immune system attacks the beta cells found in the pancreas, preventing the production of insulin. It is defined by the loss of pancreatic cells, which causes the body to produce insufficient amounts of insulin and lead to hyperglycemia (Iana and Ahmed 2023).This condition is typically present from birth or develops early in life.

  It is thought to result from an autoimmune reaction in which the beta cells, which produce insulin in the pancreas are destroyed. Before any symptoms manifest, the process may continue for months or even years (Centers for Disease Control and Prevention, 2022).

- **Type 2 Diabetes**: This occurs when the body becomes resistant to insulin, meaning the cells no longer respond effectively to it and thus instead of being absorbed by the cells, glucose remains in the blood stream (UVA Health 2025). It is the more common type of diabetes. Common symptoms include increased thirst, frequent urination, unexplained weight loss and fatigue (National Institution of Diabetes and Digestive and kidney diseases, 2014). It primarily occurs as a result of obesity and lack of exercise. It makes up about 90% of diabetes cases. If blood sugar levels are not adequately lowered, the medication, *metformin*, is typically recommended (Marthur *et al* 2016).

Ocimum gratissimum is primarily known for its potential in managing **Type 2 Diabetes Mellitus**. This is because Type 2 diabetes involves insulin resistance and elevated blood sugar levels, and *O. gratissimum* has been shown in various studies to:
- Inhibit carbohydrate-digesting enzymes like α-amylase and α-glucosidase
- Enhance insulin sensitivity
- Reduce blood glucose levels
- Exhibit antioxidant properties that protect pancreatic β-cells from oxidative stress

These mechanisms align more with the pathology of Type 2 rather than Type 1 diabetes, which is autoimmune in nature (Okoduwa *et al.,* 2017).

1.9.3 ANTI-DIABETIC ASSAY

Diabetes has caused a major burden to the health sector in the developing countries and has shown an increasing trend among the urban population (Wickramaratne *et al.,* 2016). Anti-diabetic studies are studies that investigate new drugs, compounds and natural products for their ability to manage diabetes by lowering blood glucose levels, stimulating insulin secretion and protecting organs from damage.

There are several different classes of diabetes medications that work in different ways to aid the management of blood sugar levels. They include:

- Alpha- Amylase Inhibition assay
- Alpha-glucosidase inhibitors
- Biguanides

- Bile Acid Sequestrants (BASs)
- Dopamine -2 agonists
- DPP-4 Inhibitors (gliptins)
- Meglitinides (glinides)
- Thiazolidinediones (TZDs)

Alpha Amylase Inhibition Assay: a-Amylase is a key enzyme in carbohydrate metabolism. It catalyzes the hydrolysis of alpha 1,4- glucan linkage in starch and related polysaccharides to produce oligosaccharides. a-Amylase is normally produced and stored in salivary glands and pancreas and secreted into the digestive tract. Alpha amylase inhibitors (e.g Acarbose, miglitol and voglibose) help in the prevention and treatment of metabolic syndromes such as diabetes and obesity. The assay is carried out using porcine alpha amylase and 2-chloro-4-nitrophenyl-α-D-maltotrioside as a chromogenic substrate. Upon hydrolysis by the enzyme the substrate turns to yellow colored product which can be measured colorimetrically and correlated to enzyme activity.

Alpha Glucosidase Inhibitors: The membrane-bound α-glucosidase enzyme, localized in the epithelium of small intestine hastens the digestion of oligosaccharides and disaccharides into simple glucose, after which it gets absorbed and enter into the bloodstream (AG. HB 1994). Inhibition of α-glucosidase enzyme can help in delaying digestion of carbohydrates, thereby reducing the levels of glucose in blood (Van de Laar et al 2006).

Biguanides: Biguanides are a class of oral antidiabetic agents, with metformin (1,1-dimethylbiguanide) being the primary drug currently in use. These compounds exert their glucose-lowering effects mainly by inhibiting hepatic gluconeogenesis, reducing intestinal glucose absorption, and improving peripheral insulin sensitivity (Top *et al.,* 2022). Recent insights suggest they also act via modulation of the gut microbiota and redox mechanisms in addition to classical AMPK-related pathways. Their favorable safety profile and

cardiovascular benefits have reinforced metformin's role as the first-line pharmacotherapy for type 2 diabetes mellitus. However, caution remains warranted in patients with advanced renal impairment due to the risk of lactic acidosis (Silverii *et al.,* 2024).

Bile Acid Sequestrants (BASs): Bile Acid Sequestrants (BAS) are a class of non-absorbable, polymeric drugs used primarily to lower LDL (bad) cholesterol levels in the blood. They work by binding bile acids in the intestines, preventing their reabsorption. This forces the liver to convert more cholesterol into bile acids, thereby reducing circulating cholesterol levels. Apart from treating hypercholesterolemia, some BAS (like cholestyramine) are also used to manage pruritus in liver disease and certain cases of diarrhea. (Examples include Cholestyramine, Colestipol and Colesevelam) (Feingold *et al.,* 2024).
They are generally well-tolerated but may cause gastrointestinal side effects like bloating or constipation and can interfere with the absorption of fat-soluble vitamins and some medications (Gether *et al.,* 2024).

Dopamine -2 agonists: Dopamine-2 (D2) Agonists are a class of drugs that stimulate dopamine D2 receptors in the brain. They are primarily used to treat conditions like Parkinson's disease, hyperprolactinemia, and, more recently, type 2 diabetes. In diabetes management, bromocriptine-QR, a quick-release formulation, is the main D2 agonist approved for use. It helps improve glycemic control by resetting the circadian rhythm and improving hypothalamic dopamine activity, which influences insulin sensitivity and glucose metabolism. Its glucose-lowering effects are modest and work independently of insulin secretion. Some Key benefits include:
- Low risk of hypoglycemia
- Can be combined with other antidiabetic agents
- Also beneficial for patients with cardiovascular risks
It's Common side effects include nausea, dizziness, and fatigue, especially when treatment begins (DeFronzo *et al*., 2012).

DPP-4 Inhibitors (gliptins): DPP-4 Inhibitors (Dipeptidyl Peptidase-4 inhibitors) are a class of oral antidiabetic drugs used in the management of type 2 diabetes mellitus. They work by blocking the DPP-4 enzyme, which normally breaks down incretin hormones like GLP-1 and GIP. These hormones increase insulin secretion and decrease glucagon release in a glucose-dependent manner (Gallwitz B. 2019). By inhibiting DPP-4, these drugs prolong incretin activity, leading to improved blood glucose control, especially postprandial glucose levels. They are weight-neutral and have a low risk of hypoglycemia.

Meglitinides (glinides): Meglitinides are a class of oral antidiabetic drugs used primarily to manage type 2 diabetes mellitus. They stimulate rapid, short-acting insulin secretion from the pancreatic beta cells by closing ATP-sensitive potassium channels, similar to sulfonylureas but with a faster onset and shorter duration of action (Al-Dosari *et al.,*2022).

This makes meglitinides especially effective at reducing postprandial blood glucose spikes. They are taken before meals and offer flexibility in dosing, which can help reduce the risk of hypoglycemia compared to longer-acting secretagogues. Common examples are Repaglinide and Nateglinide.

Thiazolidinediones (TZDs): Thiazolidinediones (TZDs) are oral antidiabetic drugs that improve insulin sensitivity by activating peroxisome proliferator-activated receptor gamma (PPAR-γ) in adipose tissue, muscle, and the liver. This activation enhances glucose uptake and reduces insulin resistance, making TZDs effective for managing type 2 diabetes. Common examples include pioglitazone and rosiglitazone. Despite their benefits, TZDs may cause weight gain, fluid retention, and increased risk of heart failure, so their use requires careful monitoring (Zaki *et al.,* 2023)

GC-MS

Gas Chromatography is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. It is the process of separating a mixture of compounds by injecting a gaseous or liquid sample into a mobile phase, usually an inert gas like helium or argon, and passing it through a stationary phase (might be either a liquid or a solid).

Mass spectrometry is an analytical technique that is used to measure the mass-to-charge ratio of ions of one or more molecules in the sample. It is used to determine the precise molecular weight of sample components and indirectly prove the identity of isotopes.

Gas Chromatography Mass Spectrometry (**GC-MS**) combines the two analytical techniques to a single method of analyzing a mixture of chemical compounds to identify different substances within a test sample (Sparkman *et al., 2011).* GC separates the components of the mixture and MS analyzes each of the components separately.
The GC-MS Analytical method can be used for the analysis of the obtained extracts and can be an interesting tool bf or testing the amount of some active principles in herbs used in drugs, cosmetics, food industry or pharmaceutical applications (Uma *et al*., 2009).

Fig 2.0: A Gas Chromatography Mass Spectrometer

2.0 CHAPTER TWO

2.1 MATERIALS AND METHODS

2.1.1. Materials:

The materials that were used for this study are:

Test tubes, Beakers, Conical flasks, Volumetric flasks, Measuring cylinder (10ml and 100ml), Caliberated Syringe, Pipettes, Kjeldahl flask, Funnel, Filter Papers, Spatula, Wash bottle, Glass Jar, Handkerchief, Tripod Stand, Hot plate, Oven, Furnace, Fume Cupboard, Crucible, Tongs, Electric weighing balance, Rotary Evaporator, Blender, Hot Plate, Soxhlet Apparatus, UV-Vis Spectrophotometer, GC-MS, Aluminium foil, Water bath, and an Incubator.

2.1.2. Chemicals and Reagents

Ethyl acetate, Distilled water, Conc.H_2SO_4, Sodium phosphate buffer, Ammonium molybdate, Sodium nitriprusside, Phosphate buffer saline (PBS), Griess reagent, Hexane, Alkaline Phenate, Sodium Potassium Tertarate, Sodium Hypochlorite (Bleach), Sodium sulphate, and Calcium sulphate

2.1.3. Plant Collection and Identification

Fresh stems of *Ocimum gratissimum* were obtained from a garden in Sapele road, Benin City, Edo State, Nigeria. It was identified and authenticated by Dr H.A. Akinnibosun at the herbarium unit of the Department of Plant Biology and Biotechnology, University of Benin with voucher specimen number UBH-0333.

2.2. Preparation of Extract

Fresh stems of *O. gratissimum* were rinsed, chopped into pieces and air dried for two weeks and ground to a coarsed powder with an electrical blender. The powdered stem sample weighed 180 g.

The extraction technique applied was the **maceration technique** using ethyl acetate as a solvent. The stem sample was soaked in 0.8 L of the solvent for 72 hours with intermittent stirring.

It was filtered and the filtrate concentrated using a rotary evaporator at a regulated temperature of 45^{o}C. The weight of the concentrate of the stem sample was taken to be 11.51g. And the percentage yield was calculated as;

$$\%\text{Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Sample}} \times 100$$

2.3 PROXIMATE ANALYSIS OF THE STEM OF *O. gratissimum*

The fresh,dried and ground stem sample of Ocimum gratissimum was labeled as sample A and various tests were carried out to evaluate the following: moisture content, ash content, crude protein, crude fat, crude fiber, carbohydrate content.

2.3.1 DETERMINATION OF MOISTURE CONTENT

Three grams of the sample was measure in an empty, dry, pre-weighed crucible and then placed in an air oven. The sample was dried in the oven at 110^{0}C for 3hrs. The sample was then kept in a desiccated and allowed to cool after which the crucible with the dry samples were then weighed and returned to the oven to dry for another 30 minutes to attain a constant weight (Justyna *et al*., 2025). The weights were then measured again. The moisture content was determined using the following formula:

$$\%\text{Moisture} = \frac{W_1 - W_2}{W_1 - W_0} \times 100$$

Where;

W_0 = weight of the empty crucible

W_1 = initial weight of the sample

W_2 = weight of the dry sample

2.3.2 DETERMINATION OF ASH CONTENT

Three grams of the dried sample was measured in an empty crucible and placed in a muffle furnace and heated at a very high temperature of 600^{0}C for 18hrs until a light

grey ash was obtained indicating complete combustion. The crucible was then carefully removed from the furnace using a pair of tongs and placed in a desiccator to cool. The crucible was then weighed and recorded (Ismail *et al.,* 2024). The ash content was determined by applying the formula:

$$\% \text{ Ash} = \frac{W_2 - W_0}{W_1 - W_0} \times 100$$

Where;

W_0 = weight of the empty crucible

W_1 = weight of the dry sample

W_2 = weight of the ash

2.3.3 DETERMINATION OF CRUDE PROTEIN

To estimate the crude protein content, the total nitrogen content was first determined then multiplied by a factor of 6.25. The determinant total nitrogen content was done using the Macro Kjeldahl method.

1g of the sample was measured into a conical flask and 1g each of the catalysts; sodium sulphate and calcium sulphate were added to the conical flask as well. 10ml of sulphuric acid was added to the sample and the mixture was then gently swirled to mix. It was placed on a hot plate inside a fume cupboard and allowed to digest until a clear precipitate was observed. The digested sample was then transferred into a distillation unit and 50ml of 40% NaOH was then added to make the solution strongly alkaline. A receiving flask containing boric acid + indicator (methyl red) was then positioned underneath the condenser. The mixture was distilled until 150ml of the distillate (ammonium borate) was collected (Zheng *et al.,* 2023).

The ammonium borate solution was then titrated with 0.1N HCl and the end point where the green colour changed to pink, was noted (**V**).

The total nitrogen content was then calculated by applying the formula:

$$\% \text{ Nitrogen} = \frac{V \times N \times 14.01 \times 100}{W}$$

Where **V** = titre value

N = Normality of acid used

14.01 = atomic mass of nitrogen

W = weight of sample

% Protein = % Nitrogen x Conversion factor (6.25)

2.3.4 DETERMINATION OF CRUDE FAT

The weight of a filter paper was recorded, and then 3g of the sample was measured onto filter paper. The filter paper containing the sample was then properly folded and placed in a porous thimble, which was subsequently inserted into the Soxhlet extractor. Hexane, which was used as the solvent, was heated in a round-bottomed flask positioned below the extractor. As the solvent boiled, it turned to vapour and travelled up the distillation arm into a condenser. The vapour then condensed and dripped into the thimble containing the sample.

The sample was gradually soaked with the warm solvent. As the solvent soaked the solid, the desired compounds (e.g., eugenol, thymol, and other phenolic compounds) dissolved into it, forming a solution inside the Soxhlet chamber (Zheng *et al.,* 2023).

The apparatus continued to run until the solvent in the siphon tube became clear, indicating that no more visible colour or oil was being extracted from the sample.

The samples in the thimble were then removed and dried in an oven. Once dried, their weights were taken and recorded. The difference in weight was used to calculate the percentage of the lipid(fat).

2.3.5 DETERMINATION OF CRUDE FIBER

2g of the defatted, dried plant sample ($\mathbf{W_0}$) was weighed into a beaker. To it was added 200ml of 1.25% H_2SO_4. The mixture was then boiled gently for 30 minutes. Afterwards it was carefully filtered using a wash cloth and the residue was washed repeatedly with hot water until all acid was removed.

The residue was transferred back into the beaker and 200ml of 0.313M NaOH was added and the mixture was boiled again for another 30 minutes. It was then filtered and the residue was washed with hot water and then with ethanol to remove the fat (Ramzija *et al* 2022).

The residue was then transferred to a pre weighed crucible and dried in an oven at 105^0C for 3hrs until constant weight was attained. It was cooled in a dessicator and weighed ($\mathbf{W_1}$). The crucible was then placed in a furnace at 550^0C for 3-4hrs to ash the organic matter. It was then cooled and weighed ($\mathbf{W_2}$). The percentage Crude fiber was calculated using the formula:

$$\textbf{\%Crude Fiber} = \frac{\mathbf{W_1 - W_2}}{\mathbf{W_0}} \textbf{ x 100}$$

2.3.6 DETERMINATION OF CARBOHYDRATE CONTENT

The Nittogen Free Extraction (NFE) referred to as soluble carbohydrate was not determined directly but was obtained as a sum of the percentage crude protein, crude ash, crude fat and crude fiber (Nielsen 2024).

NFE = 100% - (%Ash + %Crude fat +%Crude fiber + %Protein)

2.4. AMINO ACID INVESTIGATION OF STEM EXTRACT OF *Ocimum gratissimum.*

2.4.1. GAS CHROMATOGRAPH USED

The GC equipment consisted of Agilent 6890 technologies USA apparatus comprising an 8700 XR ternary pump, a 20-µL Rheodyne (Cotati, CA) injection loop, an SP8792 column heater, a FID detector, and a 4290 integrator linked via Labnet to a computer running WINner 8086 software (operating system, MS.DOS version 3.2). For separation, a 250- × 4.6-mm column packed with 5-µm Spherisorb C_{18} (Sugelabor, Madrid, Spain) was used.

2.4.2 PREPARATION OF SAMPLES AND STANDARDS

A 0.1g lyophilized sample was weighed into a 16 × 125 mm screw-cap tube, 15 mL of 6N hydrochloric acid was added, and the tube was throughly flushed with N_2, quickly capped, and placed in an oven at 110°C for 24hrs. After hydrolysis, the tube contents were filtered to remove solids. The filtrate was made up to 25 mL with pyridine, and an aliquot of this solution was further filtered through a 0.50-µm pore-size membrane. A standard solution containing 1.25 µmol/mL of each amino acid in 0.1M hydrochloric acid was created.

2.4.3. DERIVATIZATION PROCEDURE

The procedure used was a modification of the method of (Elkin *et al* 1987). A standard solution (5, 10, 15, or 20 µL) or 50 µL of sample solution was pipetted into a 10 × 5mm tube and dried in vacuum at 65°C. To the residue, 30 µL of methanol-water-Phenylisothiocianate (2:2:1 [v/v]) was added and then removed in vacuo at 65°C.
Next, 30µL of the derivatizing reagent methanol-water-Phenylisothiocianate (7:1:1:1 [v/v]) was added, and the tube was agitated and left to stand at room temperature for 20 min. Finally, the solvents were removed under a nitrogen stream, and the tube was sealed and stored at 4°C, pending analysis. Prior to injection, 150 µL of diluent consisting of 5mM sodium phosphate with 5% acetonitrile was added to each tube.

2.4.4. CHROMATOGRAPHIC PROCEDURE

Chromatography was carried out at a constant temperature of 30°C using a gradient elution as follows:

- Eluant A was an aqueous buffer prepared by adding 0.5 mL/L Triethylamine to 0.14M sodium acetate and titrating it to pH 6.20 with glacial acetic acid.
- Eluant B was acetonitrile-water (60:40 [v/v]).

2.5. DETERMINATION OF *IN VITRO* ANTIOXIDANT ACTIVITY ON THE ETHYL ACETATE STEM EXTRACT OF *Ocimum gratissimum*.

2.5.1. MEASUREMENT OF THE TOTAL ANTIOXIDANT CAPACITY (TAC) OF SAMPLE EXTRACTS

The Total Antioxidant Capacity (TAC) of the stem extract in different extracting solvents (absolute ethanol, 70% and 50% ethanol) were determined by the phosphomolybdate method according to Jayaprakasha *et al*., (2002).

An aliquot (30ml) of different concentrations (20,40,60,80 and 100mg/ml) of the stem extracts were mixed with 3ml of the reagent solution (0.6 M Sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) taken in test tubes. The tubes were then capped with aluminium foil and incubated in a boiling water bath at 95ºC for 90 minutes. The reaction mixture was then allowed to cool to room temperature and the absorbance of the solutions were measured at 695nm against a blank containing 3ml of reagent solution (0.6 M Sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) and the appropriate volume of the dissolved solvents. The blank was incubated under the same conditions as the test samples. Ascorbic acid was used as a standard reference to compare the activities of the extracts. The results are expressed as Ascorbic Acid Equivalent (AAE) and it is derived by plotting a calibration curve using the standard antioxidant (ascorbic acid) by plotting Absorbance (y-axis) against concentration (x-axis). From the equation of the line, **"y=mx +c"**, the absorbance of the sample is compared to the absorbance of the standard, giving the AAE.

2.5.2 MEASUREMENT OF THE NITRIC OXIDE (NO) SCAVENGING ANTIOXIDANT ASSAY OF SAMPLE EXTRACTS

The extent of inhibition of Nitric Oxide radical generation in vitro was followed as per the method reported by (Sarwar *et al* 2015):

The reaction was initiated by adding 0.5 ml of the samples (50mg), 2 ml of sodium nitroprusside and 0.5 ml of phosphate buffer saline (PBS). A control sample was also prepared by adding the same reagents but without the sample. The solutions were placed in an incubator at 25^0C for 30minutes. 0.5 ml of Greiss reagent (a mixture of sulfanilamide, N-1-naphthylethylenediamine dihydrochloride <NED>, and an acid, such as phosphoric acid) was added to each test tube and incubated again for another

30 minutes. The absorbance of the samples was then measured in a spectrometer at 546 nm. The percentage NO Scavenging activity was calculated by applying the formula:

$$\%\text{NO SCAVENGING} = \frac{\text{Abs of Control} - \text{Abs of Sample}}{\text{Abs of Control}} \times 100$$

2.6 DETERMINATION OF ANTI DIABETIC ACTIVITY OF THE ETHYL ACETATE STEM EXTRACT OF *O. GRATISSIMUM*

The Anti-diabetic properties of the plants extracts were assayed using two standard techniques.

2.6.1. Alpha Amylase Inhibitory Test

The alpha-amylase inhibitory test was performed using the standard method with minor modification (Ademiluyi *et al*., 2013). A volume of 250µl of extract or acarbose (1-300 mg/ml) was mixed with 250 µl of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase at a concentration of 0.5 mg/ml. The mixture was pre-incubated at for 10 minutes. Then, 250 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added and incubated at 25° C for another 10 minutes. The reaction was stopped by adding 500µl of dinitrosalicylic acid (DNS). The tubes were then incubated in a water bath at 95°C for 5 minutes and cooled at room temperature followed by dilution with 5 ml distilled water. The optical density was measured at 540 nm. The inhibitory activity on alpha amylase was calculated as percentage inhibition, using the following formula:

$$\%\ \text{Inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

2.6.2. Alpha Glucosidase Inhibitory Test

The ability of extracts to inhibit the activity of α-glucosidase was assessed according to (Shai *et al*., 2011). A reaction mixture containing 50 µl phosphate buffer (20 mM, pH 6.9), 10 µl α-glucosidase (1 µ/ml), and 20 µl of varying concentrations of extracts (0.0, 40.0, 80.0, 120.0 mg/ml) was incubated at 37°C for 20 minutes. Then, 20 µl P-nitrophenylglucopyranoside substrate solution (pNPG, 3mM) was added and incubated further at 37°C for another 20 minutes. The reaction was stopped with 1 ml of Na_2CO_3

(1M). The α-Glucosidase activity was determined by measuring the absorbance of the released p-nitrophenol from pNPG at 405 nm. Without test samples was set up as a control. The percentage inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{1 - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

2.7 GC-MS ANALYSIS OF OCIMUM GRATISSIMUM STEM EXRACT

The GC-MS analysis was carried out using an Agilent Technologies GC System equipped with a mass selective detector. Separation was achieved using a HP-5MS Capillary column (30m x 0.25mm i.d., 0.25um film thickness). The carrier gas used was helium at a constant flow rate of 1.0 ml/min; injection volume of 1.0ul (split ratio 10:1), injector temperature of 250ºC, Initial Oven temperature of 60ºC which was held for only 2 minutes then increased to 280ºC for 10 minutes.

The ionization mode of the mass spectrometer had an Electron Impact of 70eV with a mass scan range of: m/z 50-600. The source temperature was 230ºC and the quadrupole temperature was 150ºC

3.0 CHAPTER 3

3.1 RESULTS AND DISCUSSION

The results obtained from the proximate analysis, amino acid profile, antioxidant activities, anti diabetic activities and GC-MS analysis of the extract from the stems of *Ocimum gratissimum* are shown in the format below:

From the formula

Equation 1: $\%\text{Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Sample}} \times 100$

The yield obtained was **6.39%.**

3.2 Proximate Analysis of the Stems of Ocimum gratissimum

Table 1: Proximate (Nutritional) constituents of *O. gratissimum* stem sample.

S/N	PARAMETERS	PERCENTAGE %
1	MOISTURE	7.40
2	ASH	6.27
3	CRUDE PROTEIN	12.26
4	CRUDE FAT	4.92
5	CRUDE FIBER	12.50
6	CARBOHYDRATE	53.69

The proximate analysis of *O.gratissimum* stem in percentages (%) shown in ***Table 1***, revealed the % moisture content to be 7.40 which falls within the range of some herbs in agreements with that reported by (Nkole and Ngozi 2020). This value tells the amount of water retained either from the plant material itself or absorbed from the environment during processing or storage. Moisture content is a critical parameter in evaluating the purity and safety of herbal products. A low moisture content (typically below 10%) (Zhao *et al.,* 2021) is the ideal moisture content range dry enough to prevent spoilage, but not so dry that it affects the quality (Poo´s, *et al*., 2017). It reduces the risk of microbial growth, enzymatic activity and chemical degradation. The result suggests that the stem extract was dried properly and is relatively stable under normal storage conditions. It enhances shelf life and ensures that bioactive compounds remain intact without rapid deterioration.

In conclusion, a 7.40% moisture content indicates that the *Ocimum gratissimum* stem extract is stable, of good quality, and suitable for further pharmacological use. Low moisture content ranges from below 10% (Uddin *et al* 2011). A plant that has lower moisture content tends to have better shelf life.

The % ash content of 6.27 indicates a moderate to rich mineral composition, which is considered nutritionally valuable, especially for medicinal or dietary applications of the plant (Pandey and Sandeep, 2017). This value implies that the stem of *Ocimum gratissimum* can contribute to the mineral requirements of the body when used in herbal supplements. Minerals are important co-factors in many biochemical reactions, and their presence enhances the therapeutic potential of herbal extracts. High ash values (greater than 10%) usually indicate contamination, however the experimentally obtained ash content value obtained suggests purity and cleanness of the sample.

The % crude protein value of 12.26 indicates a moderate level of protein present in the plant material. This result suggests that *Ocimum gratissimum* stem is a potentially valuable source of plant based protein, contributing significantly to its nutritional profile. The value obtained falls between the acceptable range of 5-15% in plant stems in agreement with Martha *et al.,* 2019. The presence of protein not only supports its dietary relevance, but may also relate to biological activities such as antioxidant and enzyme inhibitory properties. Proteins and amino acids can play roles in modulating blood glucose levels and neutralizing free radicals. The protein content also suggests its potential application in herbal formulations where protein enrichment is desired.

The % crude fat of 4.92% indicates a moderate level of lipid content. The result implies that the stem contains a modest yet significant amount of lipophilic compounds. These fats are not only a source of energy but also play important roles in cell membrane structure, hormonal balance, and the adsorption of fat-soluble vitamins (A, D, E and K). Furthermore, the presence of fatty components contributes to the bioactivity of the plant, particularly in antioxidant and anti-inflammatory functions, due to the fact that many essential fatty acids are known to modulate oxidative stress and metabolic pathways. The value suggests that although the stem is not a high fat source, its lipid content may contribute meaningfully to the therapeutic and nutritional properties of *Ocimum gratissimum,* especially in herbal applications (Olawale and Adepoju, 2020).

The % crude fibre content of 12.50 indicates a high dietary fibre presence in the plants stem. A value of 12.50 suggests that the stem is a rich source of roughage, which is significant for maintaining a healthy digestive function (Akinmoladun *et al.,* 2021). Dietary fibre promotes gut motility, supports bowel regularity, and plays a key role in

preventing constipation, obesity, and colorectal diseases. It also aids in the regulation of blood sugar and cholesterol levels, making it especially beneficial for individuals managing metabolic disorders like diabetes. In medicinal plants, high fiber content may also contribute to the slowing down of glucose absorption, supporting the plants ant diabetic potential.

The % carbohydrate content of 53.69 indicates that carbohydrates constitute the major macronutrient in the stem. A value of 50% and above signifies a rich energy reserve, which could support the nutritional role of the stem in both human and animal diets. In traditional medicine, carbohydrate rich plant materials are used to support metabolic balance, and provide quick energy especially in periods of fatigue or illness (Akinmoladun *et al.,* 2021).

3.3 AMINO ACID PROFILE OF ETHYL ACETATE STEM EXTRACT OF *Ocimum gratissimum*

Table 2: Essential Amino Acid profile of stem extract of *O. gratissimum*

COMPONENT	CONCENTRATION (g/100g)
ISOLEUCINE	**2.94**
THREONINE	**1.32**
VALINE	**0.54**
LYSINE	**0.30**
GLUTAMATE	**0.29**
LEUCINE	**0.25**
PHENYLALANINE	**0.21**
TRYPTOPHAN	**0.13**
MEHIONINE	**0.08**

Table 3: Non Essential Amino Acid profile of stem extract of *O. gratissimum*

COMPONENT	CONCENTRATION (g/100g)
ALANINE	**2.26**
SERINE	**1.25**
PROLINE	**1.13**

ARGININE	**0.75**
GLUTAMATE	**0.29**
ASPARTATE	**0.20**
CYSTINE	**0.16**
TYROSINE	**0.06**
GLYCINE	**0.03**

The amino acid profile of *O. gratissimum* stem extract was analysed to assess its nutritional quality and potential health benefits. The results in Table 2 revealed a diverse composition of both essential and non-essential amino acids, contributing to its overall nutritional and therapeutic value. A thorough review of existing literature revealed a gap in research on the amino acid profile of *O. gratissimum* stem bark extract, making this study a pioneering effort in this regard.

Essential amino acids are the amino acids that the body cannot synthesize on its own, and therefore need to be ingested from external dietary sources, while non-essential amino acids are the amino acids that are already naturally present in the body and do not require external ingestion (Wu G. 2013).

A total number of 19 amino acids were identified, with isoleucine, alanine, threonine,, serine, proline, arginine and valine being among the most abundant. Amongst these, isoleucine, threonine, and valine are classified as essential amino acids while alanine, serine, arginine and proline are non-essential.

ISOLEUCINE**:** Isoleucine is an essential amino acid present in most common proteins, sometimes comprising 2 – 10% by weight. It plays several important biological roles, especially in muscle metabolism and energy regulation (Layman and Walker 2006). It is a branched-chain amino acid (BCAA), crucial for muscle protein synthesis, repair of damaged muscle tissue and promoting muscle endurance during physical activity. It helps in glucose uptake into cells and the regulation of blood sugar levels. It is also important in maintaining nitrogen balance in the body, which is essential for proper growth and body maintenance.

THREONINE: Threonine is an essential amino acid that is both glucogenic and ketogenic serving as a precursor for glycine synthesis and playing a critical role in protein synthesis and various physiological functions in mammals. A metabolomics study of human beings linked high threonine levels with longevity as defined by attaining up to 80 years (Copes *et al.,* 2015). A deficiency of threonine in mammals has been associated with depression and neurological dysfunction (Titchenal *et al.,* 1980).

VALINE: Valine is an essential amino acid obtained from the hydrolysis of proteins. It is synthesized in plants and microorganisms from pyruvic acid (Reddy and Michael., 2025). It is non polar and hydrophobic. It is crucial for metabolic functions, and its functions are vital for growth, recovery and overall physiological performance, especially in athletic contexts and during physical stress. Valine can modulate gut micro biota and immune functions. It is important for tissue recovery after illness or surgery.

ALANINE: Alanine is a non-essential amino acid, meaning the body can synthesize it on its own. It has a natural sweet taste that is used as a food and pharmaceutical additive (Mozzi 2016). It plays a central role in the glucose-alanine cycle, which transports nitrogen and carbon from muscle to the liver (Nelson and Cox 2005). It contributes to the production of antibodies and supports the function of immune cells, especially during stress or illness. The deamination reaction of alanine yields a free radical CH_3CHCO_2. This is achieved by the homolytic cleavage of the carbon-nitrogen bond due to radiation. This chemical property arising out of the alanine structure is used extensively in radiotherapy.

SERINE: Serine is a non-essential amino acid. Serine metabolism can increase cellular antioxidant function through several different mechanisms (Clare-Ann and Patrick 2019). Serine is an essential building block of lipids such as phosphatidylserine, sphingolipids and ceramides. A deficiency in the amino acid Serine, results in mitochondrial fragmentation and dysfunction (Gao *et al*., 2018).

ARGININE: Arginine is an amino acid naturally found in red meat, poultry, fish and dairy. Arginine is converted in the body into a chemical called nitric oxide. Nitric oxide causes blood vessels to open wider for improved blood flow. It also stimulates the release of growth hormones, insulin, and other substances in the body. It is used for the treatment of chest pain and various blood flow issues, erectile dysfunction, high blood pressure during pregnancy, and a serious disease in premature infants called necrotizing enterocolitis (NEC).

PROLINE: Proline is a non-essential amino acid that is synthesized from glutamate in a two-step NADPH-requiring pathway. Proline acts as a metal chelator, supports osmotic balance, and prevents oxidative damage (Hayat *et al.,* 2012). Proline supplementation mitigated the early stage of liver injury in rats by decreasing oxidative stress. It also improved redox status to improve nitric oxide availability and prevent a pathological increase in blood pressure (Leal *et al.,* 2019). It has also been shown to extend lifespan by transiently increasing ROS levels, which then stimulate pathways that induce antioxidant gene expression (Zarse *et al.,*2012).

3.4 IN VITRO ANTIOXIDANT STUDIES OF ETHYL ACETATE STEM EXTRACT RESULTS

Table 4: Phosphomolybdenum TAC assay of *O. gratissimum* stem extract

CONC of Extract (mg/ml)	Absorbance value of standard (Ascorbic acid)	Absorbance value of Sample	Ascorbic Acid Equivalent (mg/ml)
0.10	**2.01**	**18.04**	**0.31**
0.20	**2.10**	**14.92**	**0.42**
0.30	**2.18**	**19.80**	**0.52**

3.4.1. Phosphomolybdenum Method for Total Antioxidant Capacity (TAC).

The TAC assay is a laboratory test that measures the overall ability of a sample to scavenge or neutralize free radicals and other oxidants, providing an overall assessment

of its antioxidant status rather than focusing on individual antioxidants. The main goal is to assess the combined effect of all the antioxidant compounds present in the plant sample.

The Phosphomolybdenum method is a quantitative spectrophotometric assay that measures the TAC of a sample by assessing its ability to reduce **Mo(vi)** to **Mo(v).** This reduction forms a green-coloured Phosphomolybdenum (v) complex which can be quantified spectrophotometrically by measuring absorbance at 695nm. The intensity of the green colour is directly proportional to the antioxidant capacity of the sample.

Phosphomolybdenum TAC assay of *O. gratissimum* stem extract showed an appreciable increase for the standard (ascorbic acid) and the stem extract.

From the results, the antioxidant capacity of *O.gratissimum* stem extract expressed as AAE, increased with increase in concentration. The TAC of the stem extract was found to range between 0.31- 0.52 mg AAE/ml, which is considered moderate when compared to related studies on medicinal plant extracts that reported low AAE values ranging from 0.001 - 0.005 mg AAE/ml and high AAE values from 0.74 mg AAE/ml and above. (Tourabi *et al*., 2023).

These findings support the presence of potent antioxidant phytochemicals in the stem extract, making it a potential natural antioxidant source.

3.4.2. NITRIC OXIDE (NO) SCAVENGING ANTIOXIDANT ASSAY

Table 5: Nitric Oxide Scavenging Assay of stem extract of *O. gratissimum*

CONC of Extract (mg/ml)	Absorbance of extract	Absorbance of Control	%NO Scavenging
Blank	1.26	1.89	33.33
0.10	1.08	1.89	42.86
0.20	1.00	1.89	47.09
0.30	0.85	1.89	55.03

A Nitric Oxide (NO) Scavenging Assay is a laboratory method used to measure the antioxidant capacity of a substance by determining its ability to neutralize nitric oxide

radicals. The assay typically involves incubating a test sample with a source of nitric oxide (often sodium nitroprusside) and a Griess reagent (which consists of sulfanilamide and N-(1-naphthylethylenediamine) dissolved in an acidic solution, commonly phosphoric acid). The antioxidant activity is then quantified by measuring the decrease in the amount of stable nitrite product, which is detected spectrophotometrically at a specific wavelength, and calculating the percentage of NO radical inhibition.

The results presented in table 4 showed that the stem extract of *Ocimum gratissimum* exhibited nitric oxide (NO) scavenging activity at all tested concentrations (0.1, 0.2 and 0.3 mg/ml). It was observed that the % NO scavenging increased with increasing concentration, indicating a dose-dependent antioxidant response. At 0.3 mg/ml, the extract showed the highest % NO scavenging activity of 55.03 % and the lowest % NO Scavenging activity was recorded at 0.1 mg/ml with a value of 42.86%. The %NO Scavenging assay is generally considered high when it exceeds 60% and low when it is below 30% (Adebayo *et al* 2019). Overall, the stem extract demonstrated appreciable nitric oxide scavenging capacity, supporting its potential as a natural antioxidant source.

$$\%NO\ SCAVENGING = \frac{Abs\ of\ Control - Abs\ of\ Sample}{Abs\ of\ Control} \times 100$$

3.5 ANTI-DIABETIC ACTIVITY OF ETHYL ACETATE STEM EXTRACT OF *Ocimum gratissimum*

3.5.1. *IN-VITRO* α- AMYLASE INHIBITION ASSAY

Table 5: α-Amylase Inhibition Assay of stem extract of Ocimum gratissimum

CONCENTRATION (mg/ml)	ABSORBANCE OF CONTROL	ABSORBANCE OF SAMPLE (TEST)	% INHIBITION
Blank	**0.57**	**0.38**	**33.33**

40.00	**0.50**	**0.28**	**44.00**
60.00	**0.47**	**0.20**	**57.45**
80.00	**0.42**	**0.15**	**64.29**

Table 6: α-Amylase Inhibition Assay of ASCARBOSE (Standard)

CONCENTRATION (mg/ml)	ABSORBANCE OF CONTROL	ABSORBANCE OF SAMPLE (TEST)	% INHIBITION
Blank	**0.57**	**0.12**	**78.95**
40.00	**0.50**	**0.09**	**82.00**
60.00	**0.47**	**0.05**	**89.36**
80.00	**0.42**	**0.02**	**95.24**

At concentrations 40.00mg/ml, 60.00mg/ml and 80.00mg/ml, the percentage inhibition recorded were 44.00%, 57.45% and 64.29% respectively. The highest inhibition was observed at 80.00mg/ml. In comparison, Acarbose (the standard) exhibited stronger inhibitory activity, with % Inhibitions of 82.00%, 89.36% and 95.24% at the same respective concentrations.

These results suggest that while the extract demonstrated moderate α-amylase inhibition, its potency was lower than that of the standard drug. Nonetheless, the activity observed supports its traditional use in diabetes management and indicates potential for further therapeutic development.

3.5.2. Alpha Glucosidase Inhibitory Activity of *Ocimum gratissimum* Stem Extract

Table 7: α-Glucosidase Inhibition Assay of stem extract of *O. gratissimum*

CONCENTRATION (mg/ml)	ABSORBANCE OF SAMPLE	ABSORBANCE OF CONTROL	% INHIBITION
0.00	**0.51**	**0.93**	**52.69**
40.00	**0.31**	**1.10**	**62.73**

80.00	**0.27**	**1.14**	**64.04**
120.00	**0.24**	**1.17**	**64.96**
160.00	**0.19**	**1.20**	**67.50**

The α-glucosidase inhibitory potential of the stem extract of *O. gratissimum* was evaluated at concentrations ranging from 40mg/ml to 160mg/ml. The results revealed a concentration-dependent inhibition up to 160 mg/ml.

The extract showed significant inhibitory activity when compared to the control (87.10% inhibition), indicating its potential as a natural α-glucosidase inhibitor, which supports its ethno medicinal use in the management of diabetes mellitus.

The α-Amylase and α-Glucosidase Inhibition Assays were reported to be effective in the management of type 2 diabetes by targeting postprandial hyperglycemia. It was observed that inhibition of α-Amylase slows down the initial breakdown of complex carbohydrates into simpler sugars, while inhibition of α-Glucosidase delays the conversion of disaccharides to absorbable glucose. Together, these actions help reduce the rapid rise in blood glucose levels following a meal.

By moderating glucose absorption, the enzymatic inhibition approach supports glycemic control in type 2 diabetic patients, which is crucial for long term management of the condition.

3.6 GC-MS ANALYSIS RESULT OF STEM EXTRACT OF *Ocimum gratissimum*

***T*able 8: Major Compounds identified in the stem extract of *O. gratissimum* by GC-MS**

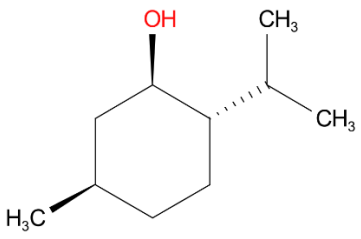
S/N	Retention Time (mins)	Compound Name	Peak Area (%)
1	**5.37**	**1-Fluorononane**	**0.61**
2	**5.47**	**Benzene, 1-ethyl-2-methyl-**	**0.99**
3	**5.62**	**1,3-cyclohexanediamine**	**0.35**

4	**5.67**	**Mesitylene (1,3,5-trimethylbenzene)**	**0.50**
5	**11.58**	**Levo-menthol**	**2.19**
6	**12.12**	**α-Terpineol**	**0.63**
7	**12.27**	**Cyclohexanol, 2-methyl-5-(1-Methylethenyl)-**	**0.96**
8	**13.02**	**Carveol (cis and trans forms)**	**0.91**
9	**13.40**	**Carveol**	**3.36**
10	**13.51**	**D-carvone**	**5.78**
11	**13.59**	**Cyclopropane**	**17.01**
12	**13.62**	**9-Borabicyclo compounds**	**28.30**
13	**14.84**	**Bicyclo [4.1.0] heptane, cyclohexanol derivatives**	**0.45**
14	**16.82**	**Cyclohexen-1-ol, acetate derivatives**	**0.70**
15	**18.22**	**Caryophyllene**	**0.26**
16	**22.76**	**Hexadecane**	**0.24**
17	**22.90**	**Dodecanoic acid (Lauric acid)**	**0.48**
18	**23.32**	**Epibubenol, Dodecanoic acid**	**0.53**
19	**29.54**	**Hexadecanoic acid, methyl ester (Methyl palmitate)**	**0.90**
20	**31.11**	**Octadecadienoic acid methyl esters (Linoleic acid derivatives)**	**0.34**
21	**31.16**	**Octadecenoic acid methyl ester (Methyl oleate)**	**2.90**
22	**31.34**	**Methyl stearate**	**0.78**
23	**31.64**	**Isopropyl tetracosyl ether, Nonyl ether derivatives**	**2.26**
24	**31.69**	**Tert-Hexadecanethiol, Octadecenoic acid derivatives**	**0.73**
25	**31.77**	**1-Nonadecene, 9-Tricosene**	**3.75**
26	**31.87**	**Cis-Vaccenic acid, 9-Octadecenoic acid, Ethanol esters**	**1.06**

27	**31.93**	**Oleic acid, ethanol esters, Cyclopropane derivatives**	**2.65**
28	**32.91**	**1-Docosene, 9-Tricosene**	**0.06**
29	**33.74**	**Oleic acid, Vaccenic acid, Cyclohexane derivatives**	**8.90**
30	**33.98**	**9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester**	**0.46**
31	**33.90**	**13-Octadecenoic acid esters**	**0.36**
32	**33.94**	**Oleic acid, Bis(2-ethylhexyl) phthalate**	**1.75**
33	**36.04**	**Erucic acid, cis-Vaccenic acid, Oleic acid**	**4.59**
34	**36.10**	**9-Octadecenoic acid esters, Oleic acid**	**0.54**
35	**36.10**	**13-Octadecenoic acid**	**3.72**

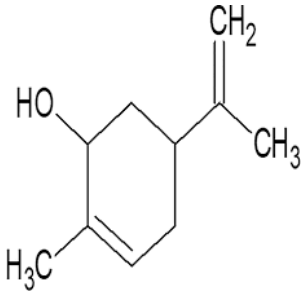
The GC-MS analysis of the methanolic stem extract of *Ocimum gratissimum* revealed a diverse array of 35 phytochemical constituents, encompassing monoterpenes, sesquiterpenes, fatty acids, esters, alcohols, and hydrocarbons. These compounds collectively contribute to the plant's traditional medicinal applications, particularly its antioxidant, anti-inflammatory and antimicrobial properties.

LEVO-MENTHOL: Levo-menthol is a naturally occurring organic compound found in mint plants. It is used in medications for its cooling, soothing and pain-relieving roerties, activating cold recetors in the body to provide relief from minor muscle and joint pain, congestion and even itching.



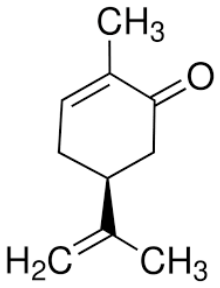
Levo-menthol

CARVEOL: Carveol is a natural unsaturated, monocyclic monoterpenoid alcohol that is a constituent of spearmint essential oil in the form of cis-**(-)**-carveol. It is a colourless fluid soluble in oils, but insoluble in water and has an odour and flavour that resemble those of spearmint and caraway. It is used as a fragrance in cosmetics and as a flavour additive in the food industry.



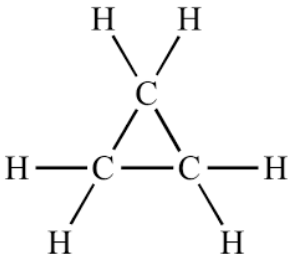
Carveol

D-CARVONE: Carvone is a member of a family of chemicals called terpenoids. Carvone is found naturally in many essential oils, but is most abundant in the oils from seeds of caraway, spearmint and dill. It serves as a flavouring agent in food products, a fragrance in perfumes and soaps, and possesses various pharmacological properties, including antidiabetic, anticancer and antimicrobial effects.



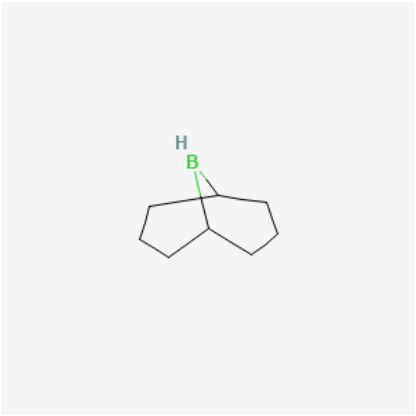
D-Carvone

CYCLOPROPANE: Cyclopropane is the cycloalkane with the formula C_3H_6, consisting of a triangular ring of 3 carbon atoms. It is an explosive, colourless gas used in medicine since 1934 as a general anaesthetic. It is not irritating to mucous membranes and does not depress respiration.



Cyclo-propane

9-BORABICYCLO COMPOUNDS: These colourless solids are used in organic chemistry as a hydroboration reagent. The compound exists as a hydride-bridged dimer, which easily cleaves in the presence of reducible substrates. An example of such compound is 9-Borabicyclo[3.3.1]nonane. This colourless solid is used in organic chemistry as a hydroboration reagent.



9-Borabicyclo[3.3.1]nonane

METHYL OLEATE: Methyl Oleate is defined as a methyl ester of oleic acid, characterized by the presence of a double bond in its aliphatic main chain, which affects its reactivity and the formation of specific products during oxidation, such as dienes and esters with multiple double bonds at high temperatures. It is used an intermediate for detergents, emulsifiers, wetting agents, stabilizers, textile treatments, plasticizers for duplicating inks, rubbers and waxes.

ISOPROPYL TETRACOSYL ETHER: This is a chemical compound with the formula $C_{27}H_{56}O$, which is an ether formed from isopropyl alcohol and a tetracosyl (24 carbon) chain. It can dissolve a range of organic compounds.

Isopropyl Tetracosyl ether

1-NONADECENE: This is an unbranched 19 carbon alkene with one double bond between C1 and C2. It has a role as a plant metabolite and a bacterial metabolite.

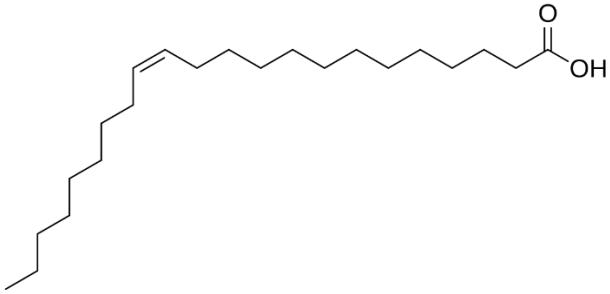
1-Nonadecene

OLEIC ACID: Oleic acid is a naturally occurring omega 9 monounsaturated fatty acid with the chemical formula $C_{18}H_{34}O_2$, found in high concentrations in foods like olive oil and animal fats. It is known for its hearthealthy roerties, helping to maintain normal cholesterol levels when used to replace saturated fats in the diet.

Oleic Acid

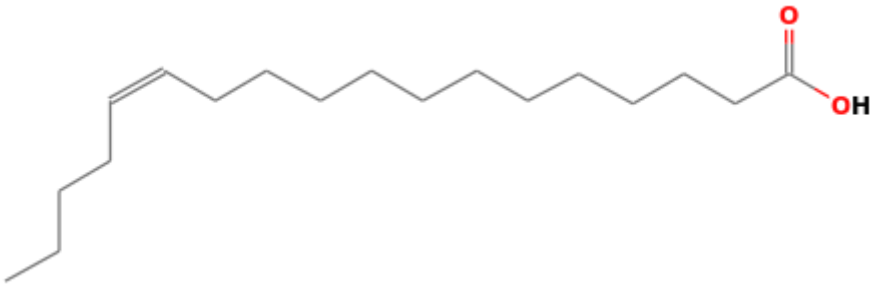
ERUCIC ACID: Erucic acid is a 22 carbon monounsaturated fatty acid. It is mainly found in oil from rape seeds, an oil that is used extensively in human foods as well as

in fish diets. It shows potential for medicinal use in neurodegenerative diseases. High, prolonged consumption of erucic acid in animal studies has been linked to myocardial lipidosis (fat deposits in the heart muscle**)**



Erucic Acid

13-OCTADECENOIC ACID: This is a long-chain fatty acid with 18 carbons and 1 double bond at position 13, existing as either a cis or trans isomer. It is a very hydrophobic molecule, especially insoluble in water, and has a relatively neutral pH.



13-OCTADECENOIC ACID

CONCLUSION

This research successfully explored the nutritional and medicinal potential of the stem extract of *Ociumum gratissimum* through a series of biochemical and phytochemical evaluations. The proximate analysis revealed that the plant stem possesses appreciable amounts of essential macronutrients, suggesting its potential contribution to dietary needs. The amino acid profiling confirmed the presence of vital amino acids required for various physiological and metabolic functions.

Furthermore, the antioxidant examinations indicated strong radical scavenging activity, highlighting the plant's potential to combat oxidative stress-related diseases. The anti-diabetic assays, particularly the inhibition of alpha-amylase and alpha-glucosidase enzymes, indicated the extracts promising role in managing postprandial hyperglycemia and by extension, Type 2 diabetes.

Finally, the GC-MS analysis revealed the presence of several bioactive comounds with known pharmacological and therapeutic properties, further supporting the traditional use of Ocimum gratissimum in herbal medicine.

Overall, the findings from this study underscore the plant's nutritional, therapeutic, and pharmacological relevance, especially in the development of functional foods and natural drug formulations. Further studies involving isolation, characterization, and in vivo examination of its bioactive components are recommended to validate and harness its full medicinal potential.

RECOMMENDATION

Based on the findings of this study, it is recommended that further in vivo and clinical studies be conducted to validate the biological activities observed in vitro, particularly the antioxidant and anti-diabetic potentials. Isolation and structural elucidation of the bioactive compounds identified through GC-MS should also be prioritized to better understand their mechanisms of action. Additionally, considering the plant's rich nutritional and phytochemical profile, its stem extract could be explored as a potential ingredient in the formulation of nutraceuticals or herbal supplements. Local

pharmaceutical and food industries are encouraged to invest in further research and development of Ocimum gratissimum as a functional natural resource.

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