GC–MS PROFILING AND *in silico* ASSESSMENT OF THE *in vitro* ANTI-PANCREATIC LIPASE ACTIVITY OF *Ocimum gratissimum* (SCENT LEAF) AQUEOUS LEAF EXTRACT

BY

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THE DEPARTMENT OF MEDICAL BIOCHEMISTRY

SCHOOL OF BASIC MEDICAL SCIENCES

UNIVERSITY OF BENIN

BENIN CITY, EDO STATE

DECEMBER, 2025

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELOR OF SCIENCE (B.Sc.) DEGREE IN MEDICAL BIOCHEMISTRY

DECEMBER, 2025

CERTIFICATION

This is to certify that this project work "GC–MS PROFILING AND *in silico* ASSESSMENT OF THE *in vitro* ANTI-PANCREATIC LIPASE ACTIVITY OF *Ocimum gratissimum* (SCENT LEAF) AQUEOUS LEAF EXTRACT" was carried out by Seth Nsikan EFFIONG with the matriculation number BMS2101391 in partial fulfillment of the award of bachelor sciences degree (B.Sc.) in the department of Medical Biochemistry, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin city, Edo state.

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DEDICATION

This project in its entirety is dedicated to God Almighty, whose grace, wisdom, and strength have been my constant source of guidance.

ACKNOWLEDGEMENTS

First and foremost, I express my profound gratitude to God Almighty for His unending grace, guidance, and strength throughout the course of this research and my academic pursuit.

My heartfelt appreciation goes to my beloved parents, Mr. and Mrs. Nsikan Effiong Tim, whose prayers, love, and unwavering support have been the backbone of my success. And to my amazing siblings Goodness, Emmanuel, Victory, and Rejoice. whose love and belief in me have been a source of strength, I am truly grateful.

I extend my sincere thanks to my project supervisor, Mr. A.E. Eguavoen, for his guidance, constructive criticism, and invaluable support which greatly contributed to the successful completion of this work. My deep appreciation also goes to the Head of Department, Dr. Bobby N. Aguebor-Ogie, for his leadership, encouragement, and academic support. I remain grateful to all my lecturers in the Department of Medical Biochemistry: Prof. (Mrs.) H. A. Oboh, Dr. J. C. Anionye, Dr. Bobby, Dr. (Mrs.) Eluehike, Dr. (Mrs.) Ikponmwonsa-Eweka, Mr. Aisosa Eguavoen, as well as all other lecturers, laboratory technologists, and non-academic staff for their dedication, mentorship, and assistance throughout my academic journey.

To everyone who contributed in one way or another to the success of this research, I express my sincere and heartfelt gratitude.

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Diabetes mellitus is a long-term metabolic condition that disrupts the normal regulation of glucose, lipid, and protein metabolism. Pancreatic lipase can contribute to diabetes by increasing the breakdown and absorption of dietary fats, which in turn raises free fatty acids in the bloodstream. These fatty acids cause lipotoxicity, damaging pancreatic β-cells and decreasing their ability to produce insulin. This study investigated the anti-pancreatic lipase potential of the aqueous leaf extract of *Ocimum gratissimum* by identifying its phytochemical constituents through Gas Chromatography-Mass Spectrometry (GC-MS) and evaluating their molecular interactions with pancreatic lipase using *in silico* techniques. Results from the study showed the presence of twenty-seven (27) phytocompounds identified from the aqueous leaf extract of *O. gratissimum* using GC-MS technique. The most abundant phytocompounds from the plant were Supraene (17.71%), Glycerin (11.38%), 1,4-Dimethoxy-2,3-dimethylbenzene (8.07%), N-Butyl acetamide (7.11%), and Thymol (5.86%). To assess their anti-pancreatic lipase potential, all twenty-seven phytocompounds and the standard drug (Orlistat) were docked with pancreatic lipase to evaluate their binding interactions with the protein. Several of the bioactive compounds demonstrated stronger binding affinity with protein in comparism with the standard. However, α-Selinene (-6.5 kcal/mol), and β-Selinene (-7.1 kcal/mol), demonstrated the strongest binding interaction with the protein when compared with the standard drug used (Orlistat) (-5.2 kcal/mol). Hydrogen bonding, salt bridge, alkyl, pi-alkyl, carbon-hydrogen bond, pi-sigma, and van der Waals were forces also observed in the study that contributed to the binding affinity of the compounds in the binding pocket of the protein. This study shows that *O. gratissimum* is a very rich source of bioactive agents that plays a significant role in the inhibition of pancreatic lipase for the attenuating of diabetes, and thus could be a strong pharmacological agent for treating diabetes.

CHAPTER ONE

1.0 INTRODUCTION

The relationship between man and medicinal plants, which has continued unabated since before recorded history, remains the bedrock of health care systems over the world (Ferreira *et al.,* 2022). Among the vast array of plant species used in traditional medicine, the genus Ocimum, which belongs to the Lamiaceae or mint family, has gained considerable recognition (Zahran *et al.,* 2020). *Ocimum gratissimum,* commonly known as Scent Leaf, African Basil, Clove Basil, or Tea Bush, is a perennial aromatic shrub and a plant of notable importance because of its characteristic pungent odor and numerous medicinal applications (Ugbogu *et al.,* 2021). Where Western medicine is indeed advanced in technology and research, it is increasingly criticized for its high cost, side effects, and symptomatic orientation rather than holistic healing (Ona *et al.,* 2022). Because traditional medicine is more accessible and cheaper, and also combined with the cultural belief system, many people, especially in developing countries, are reverting to traditional medicine (Eshete and Molla, 2021). Herbal medicines are natural therapy with less adverse effects (Salm *et al.,* 2023). *Ocimum gratissimum*, popularly known as scent leaf in Nigeria, is typical of this re-awakening. Recent studies demonstrate its anti-diabetic activity, which has been associated with its bioactive principles including eugenol, flavonoids, and tannins (Tran *et al.,* 2020). These enhance insulin secretion, improve glucose uptake, and confer protection against oxidative damage to pancreatic β-cells (Tran *et al.,* 2020). The anti-pancreatic properties, especially its antioxidant and anti-inflammatory activities, have been suggested to protect pancreatic tissue integrity and function, hence preventing the complications associated with diabetes (Gomaa *et al.,* 2024). Thus, an increased belief in medicinal plants such as *O. gratissimum* reflects both the failure of Western medicine and the rediscovery of the efficacy and sustainability of traditional healing (Manisha *et al.,* 2025).

1.1 AIM AND OBJECTIVES

The aim of this study was to investigate the anti-pancreatic lipase properties of the aqueous leaf extract of *O. gratissimum.* The specific objectives are:

i. To analyze the *in vitro* phytocompound composition of the aqueous leaf extracts of *O. gratissimum* using gas column mass spectrometry (GC-MS).

ii. To investigate the anti-pancreatic lipase activity of the aqueous leaf extract of *O. gratissimum* using *in silico* studies.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 OVERVIEW OF MEDICINAL PLANT

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times (Qadir and Raja, 2021). Plants synthesize hundreds of chemical compounds for various functions, including defense and protection against insects, fungi diseases, against parasites and herbivorous mammals (Gershenzon and Ullah, 2022). Medicinal plants are used with the intention of maintaining health, to be administered for a specific condition, or both, whether in modern medicine or in traditional medicine (Manisha *et al.,* 2025). The Food and Agriculture Organization estimated in 2002 that over 50,000 medicinal plants are used across the world (Nath *et al.,* 2023). In modern medicine, around a quarter of the drugs prescribed to patients are derived from medicinal plants, and they are rigorously tested (Chaachouay and Zidane, 2024). In other systems of medicine, medicinal plants may constitute the majority of what are often informal attempted treatments, not tested scientifically (Manisha *et al.,* 2025). The World Health Organization estimates, without reliable data, that some 80 percent of the world's population depends mainly on traditional medicine (including but not limited to plants); perhaps some two billion people are largely reliant on medicinal plants. The use of plant-based materials including herbal or natural health products with supposed health benefits, is increasing in developed countries (Bernela *et al.,* 2023). This brings attendant risks of toxicity and other effects on human health, despite the safe image of herbal remedies (Bernela *et al.,* 2023). Herbal medicines have been in use since long before modern medicine existed; there was and often still is little or no knowledge of the pharmacological basis of their actions, if any, or of their safety (Qadir and Raja, 2021). The World Health Organization formulated a policy on traditional medicine in 1991, and since then has published guidelines for them, with a series of monographs on widely used herbal medicines (Ashworth and Cloatre, 2022). Medicinal plants may provide three main kinds of benefit: health benefits to the people who consume them as medicines; financial benefits to people who harvest, process, and distribute them for sale; and society-wide benefits, such as job opportunities, taxation income, and a healthier labour force (Mabuza, 2021). However, development of plants or extracts having potential medicinal uses is blunted by weak scientific evidence, poor practices in the process of drug development, and insufficient financing (Chaachouay and Zidane, 2024).

2.2 OVERVIEW OF *Ocimum gratissimum*

O. gratissimum commonly known as African bail, is a perennial shrub native to Tropical Africa, India, and South-East Asia (Boakye *et al.,* 2023). Recognized for its culinary, ornamental uses, and medicinal properties, including management of mental illness and as a topical repellent against blackfly bites (Boakye *et al.,* 2023). *O. gratissimum* popularly known as scent leaf, 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 (Ojewumi *et al.,* 2024). It is a widespread and commercially viable perennial herbaceous plant with a very strong aromatic smell (Ojewumi *et al.,* 2024). It belongs to the family of Lamiacee and is found in Africa, Asia, and South America (Akara *et al.,* 2021). It is used as a natural flavouring agent, condiment, or vegetable in the preparation of fish, meat, soup, and stew. It is also used in traditional medicine for the treatment of several ailments such as cough, pneumonia, fever, inflammation, anaemia, diarrhea, pains, and fungal and bacterial infections (Akara *et al.,* 2021). Scientific reports have shown that *O. gratissimum* has a wide range of bioactive compounds such as flavonoids and polyphenols and essential oils with several beneficial effects (Ugbogu *et al.,* 2021).

Table 2.1 SCIENTIFIC CLASSIFICATION OF *O. gratissimum*

Kingdom	Plantae
Clade:	Tracheophytes
Clade:	Angiosperm
Clade:	Asterids
Order:	Lamiales
Family:	Lamiaceae
Genus:	*Ocimum*
Species:	*O. gratissimum*
Liberia:	Fever leaves
Edo:	Ebe-amwonkho
Yoruba:	Efirin
Igbo:	Nchanwu
Ibibio, Efik:	Ntong

Source: Ugbogu *et al.,* 2021

Figure 2.1: Image of *O. gratissimum* leaf

Source: Ugbogu *et al*., 2021

2.3 PROXIMATE ANALYSIS OF *O. gratissimum*

Ocimum gratissimum, also known as clove basil, contains a variety of phytochemicals including alkaloids, saponins, tannins, cardiac glycosides, phenols and flavonoids. These bioactive compounds also known as Phytochemicals, are associated with a range of pharmacological properties. (Ugbogu *et al.,* 2021).

1. MOISTURE

This indicates the water content, which can influence the storage life of the leaves. Range 8 - 12 % (in dried leaves); can be 60-80 % in fresh leaves (Ugbogu *et al.,* 2021).

2. ASH

This represents the total mineral content of the leaves. Range 6-9 %. A high ash content signifies that the plant is rich in minerals like calcium, potassium, magnesium and iron (Ugbogu *et al.,* 2021).

3. CRUDE PROTEIN

This indicates the amount of nitrogen-containing compounds, which are essential for building and repairing tissue. And also contributes to its medicinal values. Range 10-20% (Ugbogu *et al.,* 2021).

4. CRUDE FAT

This suggest the presence of essential oils and lipophylic compounds which are common in medicinal compounds. Range 2-6 % (Ugbogu *et al.,* 2021).

5. **CRUDE FIBRE**

This refers to the indigestible plant material, important for digestion health and suggests the plant's role in promoting bowel movement and detoxification. Range 12-18 % (Ugbogu *et al.,* 2021).

6. **CARBOHYDRATES**

This represents the major source of energy for the plant and for humans. Range 45-60% (Ademiliyi *et al.,* 2023).

2.4 PHYTOCHEMICAL COMPOSITION IN *O. gratissimum*

Phytochemicals are naturally occurring bioactive compounds produced by plants to protect themselves against pests, pathogens, and environmental stress. In humans, these compounds contribute to therapeutic activities and health benefits (Riaz *et al.,* 2023). *O. gratissimum* contain a variety of Phytochemicals including alkaloids, saponins, tannins, phenols, and flavonoids. These bioactive compounds also known as Phytochemicals, are associated with a range of pharmacological properties (Ohiagu *et al.,* 2021).

1. **ALKALOIDS**

presents in moderate amounts, possess analgesic, antimalarial and antimicrobial properties. Alkaloids are nitrogen-containing organic compounds known for their potent physiological and pharmacological activities (Aglave, 2019). In *O. gratissimum*, alkaloids such as ocimol and gratissimin have been reported. They often act on the central nervous system and cellular receptors, influencing enzyme activity, pain perception, and metabolism. The presence of alkaloids explains the use of *O. gratissimum* in relieving pain, fever, and treating malaria and bacterial infections in traditional medicine (Ugbogu *et al.,* 2021).

2. SAPONINS

Saponins are glycosides characterized by their ability to foam in aqueous solutions. Have surfactant properties, contribute to immune boosting, cholesterol lowering effects (Timilsena *et al.,* 2023). They are composed of a sugar moiety linked to a triterpene or steroidal aglycone. Exhibit antimicrobial, anti-inflammatory, antifungal, and antidiabetic properties (Rai *et al.,* 2021). Saponins reduce blood cholesterol by binding with bile acids, promote cell membrane permeability in pathogens, and modulate immune responses (Ibrahim *et al.,*2025). Saponins contribute to *O. gratissimum's* antidiabetic and antimicrobial properties and are useful in managing hyperlipidemia (Elekofehinti *et al.,* 2021).

3. TANNINS

Tannins acts as astringents and antioxidants, useful in wound healing and diarrhea treatment. Tannins are water-soluble polyphenolic compounds with astringent properties (Cosme *et al.,* 2025). They are classified as hydrolysable and condensed tannins and are present in high concentration in *O. gratissimum* leaves (Ohiagu *et al.,* 2021).

4. FLAVONOIDS

Flavonoids are a class of polyphenolic compounds widely distributed in plants. They serve as natural antioxidants and pigments responsible for coloration in leaves and flowers (Dias *et al.,* 2021). Common flavonoids in *O. gratissimum* include apigenin, quercetin, luteolin, and kaempferol. Potent antioxidant agents that neutralize free radicals and protect against oxidative stress. Exhibit anti-inflammatory, antiviral, antiallergic, and anticancer properties (Al-Khayri *et al.,* 2022). The flavonoid content contributes significantly to *O. gratissimum's* antioxidant, cardioprotective, and hepatoprotective effects.

2.5 MEDICINAL PROPERTIES OF *O. gratissimum*

Ocimum gratissimum is a perennial aromatic herb widely used in traditional medicine across Africa, Asia, and South America. Its pharmacological actions are attributed to its phytochemicals, particularly essential oils (e.g., eugenol), flavonoids, alkaloids, saponins, and phenolic compounds (Ikeotuonye *et al.,* 2023).

1. ANTIMICROBIAL ACTIVITY

The essential oils, especially eugenol, exhibit strong activity against Gram-positive and Gram-negative bacteria such as *Escherichia coli, Staphylococcus aureus*, and *Salmonella typhi*, as well as fungi like *Candida albicans* (Rasheed *et al.,* 2024). The mechanism involves disruption of microbial cell membranes and enzyme systems.

2. ANTIOXIDANT PROPERTIES

gratissimum is a powerful source of antioxidants, which are crucial in combating oxidative stress, a root cause of many chronic diseases (Oyem *et al.,* 2021). Flavonoids and phenolic compounds in *O. gratissimum* act as powerful antioxidants, scavenging reactive oxygen species (ROS) and reducing oxidative stress (Akintunde *et al.,* 2025).

3. ANTI-INFLAMMATORY EFFECTS

Extracts of the plant inhibit pro-inflammatory mediators such as prostaglandins and cytokines. Extracts reduce inflammation by inhibiting COX and LOX enzymes, thereby reducing prostaglandin and leukotriene synthesis. Flavonoids and phenolics also inhibit the NF-κB pathway, thereby reducing cytokines such as TNF-α and IL-1β (Rasheed *et al.,* 2024).

4. ANTI-DIABETIC EFFECTS

Lowers blood glucose levels, increases insulin sensitivity and glucose uptake (Akintunde *et al.,* 2025).

2.6 *O. gratissimum* AS AN ANTI-DIABETIC AGENT

Ocimum gratissimum, commonly known as scent leaf, has shown promise as an anti-diabetic agent in various studies (Ugbogu *et al.,* 2021). Extracts, particularly from the leaves, have demonstrated hypoglycemic (blood sugar-lowering) and antioxidant properties in animal models of diabetes (Ugbogu *et al.,* 2021). These findings suggest its potential in managing diabetes and its associated complications.

2.7 DIABETES MELLITUS

Diabetes mellitus, commonly known as diabetes, is a group of common endocrine diseases characterized by sustained high blood sugar levels (Povoviciu *et al.,* 2023). Diabetes is due to either the pancreas not producing enough of the hormone insulin, or the cells of the body becoming unresponsive to insulin's effects (Mann *et al.,* 2020). Classic symptoms include the three Ps: polydipsia (excessive thirst), polyuria (excessive urination), polyphagia (excessive hunger), weight loss, and blurred vision (Mann *et al.,* 2020). If left untreated, the disease can lead to various health complications, including disorders of the cardiovascular system, eye, kidney, and nerves. Diabetes accounts for approximately 4.2 million deaths every year, with an estimated 1.5 million caused by either untreated or poorly treated diabetes (Consentino *et al.,* 2020). The major types of diabetes are type 1 and type 2. The most common treatment for type 1 is insulin replacement therapy (insulin injections), while anti-diabetic medications (such as metformin and semaglutide) and lifestyle modifications can be used to manage type 2 (Tegegne *et al.,* 2024). Gestational diabetes, a form that sometimes arises during pregnancy, normally resolves shortly after delivery (Sweeting *et al.,* 2022).

2.8 TYPE 1 DIABETES

Type 1 diabetes is an autoimmune condition where the body's immune system attacks the beta cells in the pancreas, preventing the production of insulin (Roep *et al.,* 2021). This condition is typically present from birth or develops early in life. Type 1 accounts for 5 to 10% of diabetes cases and is the most common type of diabetes diagnosed in patients under 20 years; however, the older term "juvenile-onset diabetes" is no longer used as onset in adulthood is possible (Green *et al.,* 2021). The disease is characterized by loss of the insulin-producing beta cells of the pancreatic islets, leading to severe insulin deficiency, and can be further classified as immune-mediated or idiopathic (without known cause) (Infante and Ricordi, 2023). The majority of cases are immune-mediated, in which a T cell-mediated autoimmune attack causes loss of beta cells and thus insulin deficiency (Roep *et al.,* 2021). Patients often have irregular and unpredictable blood sugar levels due to very low insulin and an impaired counter response to hypoglycemia. Type 1 diabetes is partly inherited, with multiple genes, including certain HLA genotypes, known to influence the risk of diabetes (Giwa *et al.,* 2020). In genetically susceptible people, the onset of diabetes can be triggered by one or more environmental factors, such as a viral infection or diet (Zorena *et al.,* 2022). Several viruses have been implicated, but to date there is no stringent evidence to support this hypothesis in humans. Type 1 diabetes can occur at any age, and a significant proportion is diagnosed during adulthood (Tomic *et al.,* 2025). Latent autoimmune diabetes of adults (LADA) is the diagnostic term applied when type 1 diabetes develops in adults; it has a slower onset than the same condition in children (Jones *et al.,* 2021). Given this difference, some use the unofficial term "type 1.5 diabetes" for this condition. Adults with LADA are frequently initially misdiagnosed as having type 2 diabetes, based on age rather than a cause (Jones *et al.,* 2021). LADA leaves adults with higher levels of insulin production than type 1 diabetes, but not enough insulin production for healthy blood sugar levels (Giwa *et al*., 2020).

2.8.1 PREVALENCE OF TYPE 1 DIABETES MELLITUS

Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves (Giwa *et al.,* 2020). The most common is type 2 diabetes, usually in adults, which occurs when the body becomes resistant to insulin or doesn't make enough insulin (Dwivedi *at al.,* 2020). In the past 3 decades the prevalence of type 2 diabetes has risen dramatically in countries of all income levels (Quattrin *et al.,* 2023). Type 1 diabetes, once known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition in which the pancreas produces little or no insulin. There is a globally agreed target to halt the rise in diabetes and obesity by 2025. About 830 million people worldwide have diabetes, the majority living in low-and middle-income countries. More than half of people living with diabetes are not receiving treatment (Flood *et al.,* 2021). Both the number of people with diabetes and the number of people with untreated diabetes have been steadily increasing over the past decades (Flood *et al.,* 2021).

2.8.2 PATHOGENESIS OF TYPE 1 DIABETES MELLITUS

Type 1 diabetes is a result of the destruction of pancreatic beta cells, although what triggers that destruction remains unclear (Roep *et al.,* 2021). 761 People with type 1 diabetes tend to have more CD8+ T-cells and B-cells that specifically target islet antigens than those without type 1 diabetes, suggesting a role for the adaptive immune system in beta cell destruction (Flood *et al.,* 2021). Type 1 diabetics also tend to have reduced regulatory T cell function, which may exacerbate autoimmunity (Mallone and Eizirik, 2020). Destruction of beta cells results in inflammation of the islet of Langerhans, called insulitis (Smeets *et al.,* 2020). These inflamed islets tend to contain CD8+ T-cells and - to a lesser extent - CD4+ T cells. Abnormalities in the pancreas or the beta cells themselves may also contribute to beta-cell destruction (Cerf, 2020). The pancreases of people with type 1 diabetes tend to be smaller, lighter, and have abnormal blood vessels, nerve innervations, and extracellular matrix organization (Marshall, 2020). In addition, beta cells from people with type 1 diabetes sometimes over-express HLA class I molecules (responsible for signaling to the immune system) and have increased endoplasmic reticulum stress and issues with synthesizing and folding new proteins, any of which could contribute to their demise. The mechanism by which the beta cells actually die likely involves both necroptosis and apoptosis, induced or exacerbated by CD8+ T-cells and macrophages (Bertheloot *et al.,* 2021). Necroptosis can be triggered by activated T cells - which secrete toxic granzymes and perforin - or indirectly as a result of reduced blood flow or the generation of reactive oxygen species (Mázló *et al.,* 2022). As some beta cells die, they may release cellular components that amplify the immune response, exacerbating inflammation and cell death (Bertheloot *et al.,* 2021). Pancreases from people with type 1 diabetes also have signs of beta cell apoptosis, linked to activation of the janus kinase and TYK2 pathways (Chandra *et al.,* 2022). Partial ablation of beta-cell function is enough to cause diabetes; at diagnosis, people with type 1 diabetes often still have detectable beta-cell function (Atkinson and Mirmira, 2023). Once insulin therapy is started, many people experience a resurgence in beta-cell function, and can go some time with little-to-no insulin treatment - called the "honeymoon phase"(Thorne, 2025). This eventually fades as beta-cells continue to be destroyed, and insulin treatment is required again. Beta-cell destruction is not always complete, as 30-80% of type 1 diabetics produce small amounts of insulin years or decades after diagnosis (Roep *et al.,* 2021).

i. ALPHA CELL DYSFUNCTION

Onset of autoimmune diabetes is accompanied by impaired ability to regulate the hormone glucagon, which acts in antagonism with insulin to regulate blood sugar and metabolism (Haedersdal *et al.,* 2023). Progressive beta cell destruction leads to dysfunction in the neighboring alpha cells, which secrete glucagon, exacerbating excursions away from euglycemia in both directions; overproduction of glucagon after meals causes sharper hyperglycemia, and failure to stimulate glucagon upon hypoglycemia prevents a glucagon-mediated rescue of glucose levels (Masroor *et al.,* 2019).

ii. HYPERGLUCAGONEMIA

The onset of type 1 diabetes is followed by an increase in glucagon secretion after meals (Bengtsen and Moller, 2021). Increases have been measured up to 37% during the first year of diagnosis, while C-peptide levels (indicative of islet-derived insulin), decline by up to 45%. Insulin production will continue to fall as the immune system destroys beta cells, and islet-derived insulin will continue to be replaced by therapeutic exogenous insulin (Bengtsen and Moller, 2021). Simultaneously, there is measurable alpha cell hypertrophy and hyperplasia in the early stage of the disease, leading to expanded alpha cell mass (Bengtsen and Moller, 2021). This, together with failing beta cell insulin secretion, begins to account for rising glucagon levels that contribute to hyperglycemia (Hædersdal *et al.,* 2023). Some researchers believe glucagon dysregulation to be the primary cause of early-stage hyperglycemia (Liu *et al.,* 2025). Leading hypotheses for the cause of postprandial hyperglucagonemia suggest that exogenous insulin therapy is inadequate to replace the lost intraislet signalling to alpha cells previously mediated by beta cell-derived pulsatile insulin secretion (Masroor *et al.,* 2019). Under this working hypothesis intensive insulin therapy has attempted to mimic natural insulin secretion profiles in exogenous insulin infusion therapies. In young people with type 1 diabetes, unexplained deaths could be due to nighttime hypoglycemia triggering abnormal heart rhythms or cardiac autonomic neuropathy, damage to nerves that control the function of the heart (Roep *et al.,* 2021).

2.8.3 COMPLICATIONS OF TYPE 1 DIABETES

The most pressing complications of type 1 diabetes are the always-present risks of poor blood sugar control: severe hypoglycemia and diabetic ketoacidosis (Dhatariya *et al.,* 2020). Hypoglycemia - typically blood sugar below 70 mg/dL (3.9 mmol/L) - triggers the release of epinephrine, and can cause people to feel shaky, anxious, or irritable (Demirbilek *et al.,* 2023). People with hypoglycemia may also experience hunger, nausea, sweats, chills, headaches, dizziness, and a fast heartbeat (Simsek and Urhan, 2022). Some feel lightheaded, sleepy, or weak. Severe hypoglycemia can develop rapidly, causing confusion, coordination problems, loss of consciousness, and seizure (Simsek and Urhan, 2022). On average, people with type 1 diabetes experience a hypoglycemia event that requires assistance of another 16-20 times in 100 person-years, and an event leading to unconsciousness or seizure 2-8 times per 100 person-years (Chatwin *et al.,* 2021). The American Diabetes Association recommends treating hypoglycemia by the "15-15 rule": eat 15 grams of carbohydrates, then wait 15 minutes before checking blood sugar; repeat until blood sugar is at least 70 mg/dL (3.9 mmol/L). Severe hypoglycemia that impairs someone's ability to eat is typically treated with injectable glucagon, which triggers glucose release from the liver into the bloodstream (Isaacs *et al.,* 2021). People with repeated bouts of hypoglycemia can develop hypoglycemia unawareness, where the blood sugar threshold at which they experience symptoms of hypoglycemia decreases, increasing their risk of severe hypoglycemic events (Demirbilik *et al.,* 2023). Rates of severe hypoglycemia have generally declined due to the advent of rapid-acting and long-acting insulin products in the 1990s and early 2000s; however, acute hypoglycemia still causes 4-10% of type 1 diabetes-related deaths (Snaith *et al.,* 2020). The other persistent risk is diabetic ketoacidosis, a state where lack of insulin results in cells burning fat rather than sugar, producing toxic ketones as a byproduct (Elendu *et al.,* 2023). Ketoacidosis symptoms can develop rapidly, with frequent urination, excessive thirst, nausea, vomiting, and severe abdominal pain all common (Karrar *et al.,* 2022). More severe ketoacidosis can result in labored breathing, and loss of consciousness due to cerebral edema (Karrar *et al.,* 2022). People with type 1 diabetes experience diabetic ketoacidosis 1-5 times per 100 person-years, the majority of which result in hospitalization (Thomas *et al.,* 2020). 13-19% of type 1 diabetes-related deaths are caused by ketoacidosis, making ketoacidosis the leading cause of death in people with type 1 diabetes less than 58 years old (Ehrmann *et al.,* 2020).

i. LONG TERM COMPLICATIONS

In addition to the acute complications of diabetes, long-term hyperglycemia results in damage to the small blood vessels throughout the body (Mezil and Abed 2021). This damage tends to manifest particularly in the eyes, nerves, and kidneys, causing diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy, respectively (Mezil and Abed 2021). In the eyes, prolonged high blood sugar causes the blood vessels in the retina to become fragile. People with type 1 diabetes also have an increased risk of cardiovascular disease, which is estimated to shorten the life of the average type 1 diabetic by 8 to 13 years (Colom *et al.,* 2021). Cardiovascular disease as well as neuropathy, may have an autoimmune basis, as well

(Conrad *et al.,* 2022). Women with type 1 DM have a 40% higher risk of death as compared to men with type 1 DM (Schofield, *et al* 2019). About 12 percent of people with type 1 diabetes have clinical depression (Farooqi *et al.,* 2022). About 6 percent of people with type 1 diabetes also have celiac disease, but in most cases there are no digestive symptoms or are mistakenly attributed to poor control of diabetes, gastroparesis, or diabetic neuropathy (Marathe *et al.,* 2024). In most cases, celiac disease is diagnosed after the onset of type 1 diabetes. The association of celiac disease with type 1 diabetes increases the risk of complications, such as increases the risk of complications, such as retinopathy and mortality (Eland *et al.,*2022). This association can be explained by shared genetic factors, and inflammation or nutritional deficiencies caused by untreated celiac disease, even if type 1 diabetes is diagnosed first.

ii. URINARY TRACT INFECTION (UTI)

People with diabetes show an increased rate of urinary tract infection. The reason is that bladder dysfunction is more common in people with diabetes than in people without diabetes due to diabetes nephropathy (Sharma *et al.,* 2025). When present, nephropathy can cause a decrease in bladder sensation, which in turn can cause increased residual urine, a risk factor for urinary tract infections (Sharma *et al.,* 2025).

iii. SEXUAL DYSFUNCTION

Sexual dysfunction in people with diabetes is often a result of physical factors such as nerve damage and poor circulation, and psychological factors such as stress and/or depression caused by the demands of the disease (Winkley *et al.,* 2021). The most common sexual issues in males with diabetes are problems with erections and ejaculation: "With diabetes, blood vessels supplying the penis's erectile tissue can get hard and narrow, preventing the adequate blood supply needed for a firm erection (Mostafa and Abdel-Hamid, 2021). The nerve damage caused by poor blood glucose control can also cause ejaculate to go into the bladder instead of through the penis during ejaculation, called retrograde ejaculation (Mostafa and Abdul-Hamid, 2021). When this happens, semen leaves the body in the urine." Another cause of erectile dysfunction is reactive oxygen species created as a result of the disease. Antioxidants can be used to help combat this (Zhu *et al.,* 2025). Sexual problems are common in women who have diabetes, including reduced sensation in the genitals, dryness, difficulty/inability to orgasm, pain during sex, and decreased libido (Winkley *et al.,* 2021). Diabetes sometimes decreases estrogen levels in females, which can affect vaginal lubrication (O'Laughlin and McCoy, 2023). Less is known about the correlation between diabetes and sexual dysfunction in females than in males. Oral contraceptive pills can cause blood sugar imbalances in women who have diabetes (Fenasse and McEwen, 2019). Dosage changes can help address that, at the risk of side effects and complications. Women with type 1 diabetes show a higher than normal rate of polycystic ovarian syndrome (PCOS) (Thong *et al.,* 2020). The reason may be that the ovaries are exposed to high insulin concentration since women with type 1 diabetes can have frequent hyperglycemia.

iv. AUTOIMMUNE DISORDER

People with type 1 diabetes are at an increased risk for developing several autoimmune disorders, particularly thyroid problems (Popoviciu *et al.,* 2023). Around 20% of people with type 1 diabetes have hypothyroidism or hyperthyroidism, typically caused by Hashimoto thyroiditis or Graves' disease respectively (Popoviciu *et al.,* 2023). Celiac disease affects 2-8% of people with type 1 diabetes, and is more common in those who were younger at diabetes diagnosis, and in white people (Monar *et al.,* 2022). Type 1 diabetics are also at increased risk of rheumatoid arthritis, lupus, autoimmune gastritis, pernicious anemia, vitiligo, and Addison's disease (Popoviciu *et al.,* 2023). Conversely, complex autoimmune syndromes caused by mutations in the immunity-related genes AIRE (causing autoimmune polyglandular syndrome), FoxP3 (causing IPEX syndrome), or STAT3 include type 1 diabetes in their effects (Peddi *et al.,* 2023).

v. DIABETIC KETO ACIDOSIS (DKA)

Diabetic ketoacidosis (DKA) is one of the life-threatening severe complications of diabetes that demands immediate attention and intervention (Mahajan and Mahayana, 2023). It is considered a medical emergency and can affect both patients with T1D (type 1 diabetes) and T2D (type 2 diabetes), but it is more common in T1D. DKA results from significantly low insulin levels due to various factors including undiagnosed diabetes (people who did not know they have diabetes), missed or delayed doses, insufficient insulin administration, or undergoing physiological stress (e.g. infection, surgery, Stroke, or trauma) (Dhatariya *et al.,* 2020). Due to insulin absence, it simply triggers the release of counter-regulatory hormones resulting in serious health complications (Mahajan and Mahayana, 2023). This release prompts excessive free fatty acids (FFAs) production as a result of the adipose tissue exhibiting heightened activity of hormone-sensitive lipase. Subsequently, the liver turns fatty acid to ketone bodies for fuel, a process known as ketosis, which causes Ketonemia (high ketone level in the blood) that decreases the blood's pH, leading to DKA (Zhu *et al.,* 2022). While periodic ketosis is normal, but can become a serious problem if sustained. These hormones can also induce hyperglycemia (high blood glucose) by stimulating gluconeogenesis thereby increasing the renal glucose output (Zhu *et al.,* 2022). In addition to the endogenous renal ketosis process. While replacing fluid and electrolyte loss, insulin, and acid-placed balance are the aim of this treatment. Proper treatment usually results in full recovery, though death can result from inadequate or delayed treatment, or from complications (e.g., brain edema) (Zhu *et al.,* 2022). Preventing DKA is attainable by following some precautions. While feeling unwell, Start with regular monitoring of blood glucose levels (Trojanowski *et al.,* 2021). In addition to measuring blood or urine ketone concentrations twice a day and more. In case there are ketones, insulin doses should be increased. Patients are also advised to focus on dehydration and go to the hospital in case of frequent vomiting. It's essential to emphasize that insulin should never be discontinued, even if there is no intake of food or fluids. Patients' education and awareness of managing a sick day is a key element, as recognizing symptoms, and knowing when to contact a healthcare provider. This education significantly contributes to reducing the occurrence of DKA (Trojanowski *et al.,* 2021).

vi. DIABETIC FOOT ULCER

Diabetic foot ulcer is a breakdown of the skin and sometimes deeper tissues of the foot that leads to sore formation (Amstrong *et al.,* 2023). It is thought to occur due to abnormal pressure or mechanical stress chronically applied to the foot, usually with concomitant predisposing conditions such as peripheral sensory neuropathy, peripheral motor neuropathy, autonomic neuropathy or peripheral arterial disease (Parveen *et al.,* 2025). It is a major complication of diabetes mellitus, and it is a type of diabetic foot disease. Secondary complications to the ulcer, such as infection of the skin or subcutaneous tissue, bone infection, gangrene or sepsis are possible, often leading to amputation (Parveen *et al.,* 2025). A key feature of wound healing is stepwise repair of lost extracellular matrix (ECM), the largest component of the dermal skin layer. However, in some cases, physiological insult or disorder in this case, diabetes mellitus impedes the wound healing process (Paster *et al.,* 2024). In diabetic wounds, the inflammatory phase of the healing process is prolonged, delaying the formation of mature granulation tissue and reducing the healing wound's tensile strength (Cai *et al.,* 2023). Treatment of diabetic foot ulcers includes blood sugar control, removal of dead tissue from the wound, wound dressings, and removing pressure from the wound through techniques such as total contact casting (Wang *et al.,* 2021). Surgery, in some cases, may improve outcomes. Hyperbaric oxygen therapy may also help but is expensive. 34% of people with diabetes develop a diabetic foot ulcer during their lifetime, and 84% of all diabetes-related lower-leg amputations are associated with or result from diabetic foot ulcers (Hsia *et al.,* 2025).

2.8.4 DIAGNOSIS OF TYPE 1 DIABETES MELLITUS

Diabetes mellitus is diagnosed with a test for the glucose content in the blood, and is diagnosed by demonstrating any one of the following:

i. FASTING PLASMA GLUCOSE LEVEL

A fasting plasma glucose value of ≥ 7.0 mmol/L (126 mg/dL) is diagnostic, with the measurement obtained from a blood sample collected after an overnight fast or a minimum fasting period of eight hours (Sacks *et al.,* 2023).

ii. PLASMA GLUCOSE

A plasma glucose concentration ≥ 11.1 mmol/L (200 mg/dL) measured two hours after administering a 75-g oral glucose load during an OGTT, or the presence of symptoms of hyperglycaemia accompanied by a random plasma glucose ≥ 11.1 mmol/L (200 mg/dL), is considered diagnostic (Sacks *et al.,* 2023).

iii. GLYCATED HEMOGLOBIN

An HbA1c value ≥ 48 mmol/mol (≥ 6.5% DCCT) is diagnostic for diabetes; however, in the absence of unequivocal hyperglycaemia, the result should be confirmed by repeating any of the diagnostic tests on a separate day. Fasting plasma glucose measurement is generally preferred due to its simplicity and the reduced time burden compared with the oral glucose tolerance test, which requires two hours and offers no additional prognostic benefit. Under current criteria, two fasting plasma glucose results ≥ 7.0 mmol/L (126 mg/dL) on different days confirm the diagnosis of diabetes mellitus (Sacks *et al.,* 2023).

2.8.5 MANAGEMENT OF TYPE 1 DIABETES MELLITUS

Diabetes management concentrates on keeping blood sugar levels close to normal, without causing low blood sugar (van Netten *et al.,* 2024). This can usually be accomplished with dietary changes, exercise, weight loss, and use of appropriate medications (insulin, oral medications). Learning about the disease and actively participating in the treatment is important, since complications are far less common and less severe in people who have well-managed blood sugar levels. The goal of treatment is an A1C level below 7%. Attention is also paid to other health problems that may accelerate the negative effects of diabetes. These include smoking, high blood pressure, metabolic syndrome obesity, and lack of regular exercise. Specialized footwear is widely used to reduce the risk of diabetic foot ulcers by relieving the pressure on the foot (van Netten *et al.,* 2024). Foot examination for patients living with diabetes should be done annually which includes sensation testing, foot biomechanics, vascular integrity and foot structure (Dhlamini and Houreld, 2022). Concerning those with severe mental illness, the efficacy of type 2 diabetes self-management interventions is still poorly explored, with insufficient scientific evidence to show whether these interventions have similar results to those observed in the general population (Dhlamini and Houreld 2022).

i. EDUCATION

Diabetes management for children requires the integration of the family and health care team to be committed and continuous for promotion of self-management (Batista *et al.,* 2021). A health care team may include a pediatric endocrinologist or physician trained in pediatric diabetes, a diabetes specialist nurse, a registered dietitian, a psychologist, a social worker, and child life specialist. The goal of the health care team and child's family is to empower the child to make informed decisions for health-promoting lifestyle choices (Batista *et al.,* 2021).

ii. LIFESTYLE

People with diabetes can benefit from education about the disease and treatment, dietary changes, and exercise, with the goal of keeping both short-term and long-term blood glucose levels within acceptable bounds (Joseph *et al.,* 2022). In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications are recommended to control blood pressure (Samuel *et al.,* 2024). Weight loss can prevent progression from prediabetes to diabetes type 2, decrease the risk of cardiovascular disease, or result in a partial remission in people with diabetes (Birkenfeld and Mohan, 2024). No single dietary pattern is best for all people with diabetes. Healthy dietary patterns, such as the Mediterranean diet, low-carbohydrate diet, or DASH diet, are often recommended, although evidence does not support one over the others. According to the ADA, "reducing overall carbohydrate intake for individuals with diabetes has demonstrated the most evidence for improving glycemia", and for individuals with type 2 diabetes who cannot meet the glycemic targets or where reducing anti-glycemic medications is a priority, low or very-low carbohydrate diets are a viable approach (Nicholas *et al.,* 2021). For overweight people with type 2 diabetes, any diet that achieves weight loss is effective (Chandrasekaran and Weiskirchen, 2024).

iii. IN CHILDREN

While type 1 diabetes is more prevalent in pediatric diabetes, type 2 diabetes has increasing prevalence, accounting for some 33% of new diagnoses (ElSayed *et al.,* 2023). Risk factors for type 2 diabetes include ethnicity, family history, sedentary lifestyle, unhealthy diet, a mother with gestational diabetes, female gender, and obesity (Yang *et al.,* 2022). Children with type 2 diabetes have increased risk of developing complications, which include insulin resistance, hyperglycemia, polyuria, ketosis, and dehydration (Serbis *et al.,* 2021). Early recognition, screening, treatment, and education of diabetic children are needed to prevent long-term disease complications (Serbis *et al.,* 2021). Screening for type 2 diabetes typically starts at 10 years old for obese children and those who have at least two risk factors (Al Hourani *et al.,* 2021). Diagnostic criteria include plasma blood glucose of more than 200 mg per deciliter (di) or a fasting blood glucose above 126 mg per di in children with overt symptoms (Al Hourani *et al.,* 2021). Differentiating type 1 from type 2 diabetes may include assessment of fasting blood insulin or C-peptide, or determination of autoantibodies for type 1 diabetes (Iqbal *et al.,* 2023).

iv. GLUCOSE CONTROL

Most medications used to treat diabetes act by lowering blood sugar levels through different mechanisms (Ahmed and Khalique, 2020). There is broad consensus that when people with diabetes maintain tight glucose control, keeping the glucose levels in their blood within normal ranges they experience fewer complications, such as kidney problems or eye problems (Ahmed and Khalique, 2020). There is, however, debate as to whether this is appropriate and cost effective for people later in life in whom the risk of hypoglycemia may be more significant.

v. BLOOD PRESSURE LOWERING

Cardiovascular disease is a serious complication associated with diabetes, and many international guidelines recommend blood pressure treatment targets that are lower than 140/90 mmHg for people with diabetes (Kim and Kim, 2022). However, there is only limited evidence regarding what the lower targets should be. A 2016 systematic review found potential harm to treating to targets lower than 140 mmHg, and a subsequent systematic review in 2019 found no evidence of additional benefit from blood pressure lowering to between 130 - 140mmHg, although there was an increased risk of adverse events (Saiz *et al.,* 2022). The 2015 American Diabetes Association recommendations are that people with diabetes and albuminuria should receive an inhibitor of the renin-angiotensin system to reduce the risks of progression to end-stage renal disease, cardiovascular events, and death (de Boer *et al.,* 2022). There is some evidence that angiotensin converting enzyme inhibitors (ACEIs) are superior to other inhibitors of the renin-angiotensin system such as angiotensin receptor blockers (ARBs), or aliskiren in preventing cardiovascular disease (Omboni and Volpe, 2019). Although a more recent review found similar effects of ACEIs and ARBs on major cardiovascular and renal outcomes. There is no evidence that combining ACEIs and ARBs provides additional benefits.

2.9 TYPE 2 DIABETES

Diabetes mellitus type 2, commonly known as type 2 diabetes (T2D), and formerly known as adult-onset diabetes, is a form of diabetes mellitus that is characterized by high blood sugar, insulin resistance, and relative lack of insulin (Misra *et al.,* 2023). Common symptoms include increased thirst, frequent urination, fatigue and unexplained weight loss. Other symptoms include increased hunger, having a sensation of pins and needles, and sores (wounds) that heal slowly (Misra *et al.,* 2023). Symptoms often develop slowly. Long-term complications from high blood sugar include heart disease, stroke, diabetic retinopathy, which can result in blindness, kidney failure, and poor blood flow in the lower limbs, which may lead to amputations (Salvatore *et al.,* 2023). " A sudden onset of hyperosmolar hyperglycemic state may occur; however, ketoacidosis is uncommon (Salvatore *et al.,* 2023). Type 2 diabetes primarily occurs as a result of obesity and lack of exercise." Some people are genetically more at risk than others (Pillon *et al.,* 2021). Type 2 diabetes makes up about 90% of cases of diabetes, with the other 10% due primarily to type 1 diabetes and gestational diabetes (Auvinen *et al.,* 2020). Diagnosis of diabetes is by blood tests such as fasting plasma glucose, oral glucose tolerance test, or glycated hemoglobin (A1c) (Goyal *et al.,* 2021). Type 2 diabetes is largely preventable by staying at a normal weight, exercising regularly, and eating a healthy diet (high in fruits and vegetables and low in sugar and saturated fat) (Magkos *et al.,* 2020). Treatment involves exercise and dietary changes. If blood sugar levels are not adequately lowered, the medication metformin is typically recommended (Baker *et al.,* 2021). Many people may eventually also require insulin injections. In those on insulin, routinely checking blood sugar levels (such as through a continuous glucose monitor) is advised; however, this may not be needed in those who are not on insulin therapy (Baker *et al*., 2021). Bariatric surgery often improves diabetes in those who are obese (Doumouras *et al.,* 2021). Rates of type 2 diabetes have increased markedly since 1960 in parallel with obesity (Murugan, 2021). As of 2015, there were approximately 392 million people diagnosed with the disease compared to around 30 million in 1985 (Looker *et al.,* 2020). Typically, it begins in middle or older age, although rates of type 2 diabetes are increasing in young people (Looker *et al.,* 2020). Type 2 diabetes is associated with a ten-year-shorter life expectancy (Murugan, 2021). Diabetes was one of the first diseases ever described, dating back to an Egyptian manuscript from c. 1500 BCE (Mohajan and Mohajan, 2023). Type 1 and type 2 diabetes were identified as separate conditions in 400-500 CE with type 1 associated with youth and type 2 with being overweight (Karavanaki *et al.,* 2022). The importance of insulin in the disease was determined in the 1920s.

2.9.1 PREVALENCE OF TYPE 2 DIABETES

Rates of type 2 diabetes have increased markedly since 1960 in parallel with obesity (Murugan, 2021). As of 2015, there were approximately 392 million people diagnosed with the disease compared to around 30 million in 1985 (Looker *et al.,* 2020). Typically, it begins in middle or older age, although rates of type 2 diabetes are increasing in young people (Misra *et al.,* 2023). Type 2 diabetes is associated with a ten-year-shorter life expectancy (Murugan, 2021). Diabetes was one of the first diseases ever described, dating back to an Egyptian manuscript from c. 1500 BCE (Mohajan and Mohajan, 2023). Type 1 and type 2 diabetes were identified as separate conditions in 400-500 CE with type 1 associated with youth and type 2 with being overweight (Karavanaki *et al.,* 2022). The importance of insulin in the disease was determined in the 1920s.

2.9.2 PATHOGENESIS OF TYPE 2 DIABETES

Type 2 diabetes is due to insufficient insulin production from beta cells in the setting of insulin resistance (Weir *et al.,* 2020). Insulin resistance, which is the inability of cells to respond adequately to normal levels of insulin, occurs primarily within the muscles, liver, and fat tissue (Khalilov and Abdullayeva, 2023). In the liver, insulin normally suppresses glucose release. However, in the setting of insulin resistance, the liver inappropriately releases glucose into the blood (James *et al.,* 2021). The proportion of insulin resistance versus beta cell dysfunction differs among individuals, with some having primarily insulin resistance and only a minor defect in insulin secretion and others with slight insulin resistance and primarily a lack of insulin secretion (Angelidi *et al.,* 2021). Other potentially important mechanisms associated with type 2 diabetes and insulin resistance include: increased breakdown of lipids within fat cells, resistance to and lack of incretin, high glucagon levels in the blood, increased retention of salt and water by the kidneys, and inappropriate regulation of metabolism by the central nervous system (Angelidi *et al*., 2021). However, not all people with insulin resistance develop diabetes since an impairment of insulin secretion by pancreatic beta cells is also required (Park *et al.,* 2021). In the early stages of insulin resistance, the mass of beta cells expands, increasing the output of insulin to compensate for the insulin insensitivity, so that the disposition index remains constant (Park *et al.,* 2021). But when type 2 diabetes has become manifest, the person will have lost about half of their beta cells (Reed *et al.,* 2021). The causes of the aging-related insulin resistance seen in obesity and in type 2 diabetes are uncertain. Effects of intracellular lipid metabolism and ATP production in liver and muscle cells may contribute to insulin resistance (Rizvi and Rizzo, 2024).

2.9.3 COMPLICATIONS OF TYPE 2 DIABETES

Complications of diabetes are secondary diseases that are a result of elevated blood glucose levels that occur in diabetic patients (Prabhakar, 2024). These complications can be divided into two types: acute and chronic. Acute complications are complications that develop rapidly and can be exemplified as diabetic ketoacidosis (DKA), hyperglycemic hyperosmolar state (HHS), lactic acidosis (LA), and hypoglycemia (Ghasemi-Dehnoo *et al.,* 2020). Chronic complications develop over time and are generally classified in two categories: microvascular and macrovascular (Caturano *et al.,* 2025). Microvascular complications include neuropathy, nephropathy, and retinopathy; while cardiovascular disease, stroke, and peripheral vascular disease are included in the macrovascular complications (Caturano *et al.,* 2025). The complications of diabetes can dramatically impair quality of life and cause long-lasting disability (Wroblewska *et al.,* 2023). Overall, complications are far less common and less severe in people with well-controlled blood sugar levels. Some non-modifiable risk factors such as age at diabetes onset, type of diabetes, gender, and genetics may influence risk (Jareebi and Gosadi, 2025). Other health problems compound the chronic complications of diabetes such as smoking, obesity, high blood pressure, elevated cholesterol levels, and lack of regular exercise (Sharma, *et al* 2020). Complications of diabetes are a strong risk factor for severe COVID-19 illness (Pugliese *et al.,* 2020).

i. DIABETIC KETONE ACIDOSIS (DKA)

Diabetic ketoacidosis (DKA) is one of the life-threatening severe complications of diabetes that demands immediate attention and intervention (Karrar *et al.,* 2023). It is considered a medical emergency and can affect both patients with T1D (type 1 diabetes) and T2D (type 2 diabetes), but it is more common in T1D (Karrar *et al.,* 2023). DKA results from significantly low insulin levels due to various factors including undiagnosed diabetes (people who did not know they have diabetes), missed or delayed doses, insufficient insulin administration, or undergoing physiological stress (e.g. infection, surgery, Stroke, or trauma) (Nasa *et al.,* 2021). Due to insulin absence, it simply triggers the release of counter-regulatory hormones resulting in serious health complications (Nasa *et al.,* 2021). This release prompts excessive free fatty acids (FFAs) production as a result of the adipose tissue exhibiting heightened activity of hormone-sensitive lipase. Subsequently, the liver turns fatty acid to ketone bodies for fuel, a process known as ketosis, which causes Ketonemia (high ketone level in the blood) that decreases the blood's pH, leading to DKA (Zhu *et al.,* 2022). While periodic ketosis is normal, but can become a serious problem if sustained (Zhu *et al.,* 2022). These hormones can also induce hyperglycemia (high blood glucose) by stimulating gluconeogenesis thereby increasing the renal glucose output (Legouis *et al.,* 2022). In addition to the endogenous renal glucose produced by the kidneys. The condition of high circulating concentrations of ketone bodies and hyperglycemia leads to osmotic diuresis, characterized by the excessive presence of glucose and ketones in the urine (Legouis *et al.,* 2022). Consequently, osmotic diuresis causes dehydration and electrolyte loss. Symptoms of DKA can be noticed within a few hours, like polyuria (excessive urine production), polydipsia (excessive thirst), Weight loss, weakness, nausea, vomiting, and deep rapid breathing (Kussmaul respiration) (Karrar *et al.,* 2022). Moreover, abdominal pain is common and may be severe. The level of consciousness is typically normal until late in the process, when lethargy may progress to coma. Ketoacidosis can easily become severe enough to cause hypotension, shock, and death (Karrar *et al.,* 2022). The DKA is diagnosed by the urine analysis which will reveal significant levels of ketone bodies (which have exceeded their renal threshold blood levels to appear in the urine, often before other overt symptoms) (Kilpatrick *et al.,* 2022). And also venous blood investigation for electrolytes, glucose, and acid-base status. The expected result of the treatment tackles the deeper causes; which are dehydration, acidosis, and hyperglycemia, and initiates a reversal of the ketosis process (Kilpatrick *et al.,* 2022). While replacing fluid and electrolyte loss, insulin, and acid-placed balance are the aim of this treatment (Cook *et al.,* 2020). Proper treatment usually results in full recovery, though death can result from inadequate or delayed treatment, or from complications (e.g., brain edema) (Cook *et al.,* 2020). Preventing DKA is attainable by following some precautions. While feeling unwell, Start with regular monitoring of blood glucose levels. In addition to measuring blood or urine ketone concentrations twice a day and more (Kong *et al.,* 2024). In case there are ketones, insulin doses should be increased. Patients are also advised to focus on dehydration and go to the hospital in case of frequent vomiting (Lough, 2022). It's essential to emphasize that insulin should never be discontinued, even if there is no intake of food or fluids (Lough, 2022). Patients' education and awareness of managing a sick day is a key element, as recognizing symptoms, and knowing when to contact a healthcare provider (Dhaliwal *et al.,* 2023). This education significantly contributes to reducing the occurrence of DKA.

ii. DIABETIC FOOT ULCER

Diabetic foot ulcer is a breakdown of the skin and sometimes deeper tissues of the foot that leads to sore formation (Amstrong *et al.,* 2023). It is thought to occur due to abnormal pressure or mechanical stress chronically applied to the foot, usually with concomitant predisposing conditions such as peripheral sensory neuropathy, peripheral motor neuropathy, autonomic neuropathy or peripheral arterial disease (Amstrong *et al.,* 2023). It is a major complication of diabetes mellitus, and it is a type of diabetic foot disease. Secondary complications to the ulcer, such as infection of the skin or subcutaneous tissue, bone infection, gangrene or sepsis are possible, often leading to amputation (Primadhi *et al.,* 2023). A key feature of wound healing is stepwise repair of lost extracellular matrix (ECM), the largest component of the dermal skin layer (Diller and Tabor, 2022). However, in some cases, physiological insult or disorder in this case, diabetes mellitus impedes the wound healing process (Pastar *et al.,* 2024). In diabetic wounds, the inflammatory phase of the healing process is prolonged, delaying the formation of mature granulation tissue and reducing the healing wound's tensile strength (Pastar, *et al* 2024). Treatment of diabetic foot ulcers includes blood sugar control, removal of dead tissue from the wound, wound dressings, and removing pressure from the wound through techniques such as total contact casting. Surgery, in some cases, may improve outcomes (Amstrong *et al.,* 2023). Hyperbaric oxygen therapy may also help but is expensive. 34% of people with diabetes develop a diabetic foot ulcer during their lifetime, and 84% of all diabetes-related lower-leg amputations are associated with or result from diabetic foot ulcers (Sharma, 2021).

iii. HYPOGLYCEMIA

Hypoglycemia, or abnormally low blood glucose, is an acute complication of several diabetes treatments (Mohajan and Mohajan, 2023). It is rare otherwise, either in diabetic or non-diabetic patients. The patient may become agitated, sweaty, weak, and have many symptoms of sympathetic activation of the autonomic nervous system resulting in feelings akin to dread and immobilized panic (Mohajan and Mohajan, 2023). Consciousness can be altered or even lost in extreme cases, leading to coma, seizures, or even brain damage and death. In patients with diabetes, this may be caused by several factors, such as too much or incorrectly timed insulin, too much or incorrectly timed exercise (exercise decreases insulin requirements) or not enough food (specifically glucose containing carbohydrates) (Awuchi *et al.,* 2020). The variety of interactions makes cause identification difficult in many instances. It is more accurate to note that iatrogenic hypoglycemia is typically the result of the interplay of absolute (or relative) insulin excess and compromised glucose counter regulation in type 1 and advanced type 2 diabetes (Monnier *et al.,* 2025). Decrements in insulin, increments in glucagon, and, absent the latter, increments in epinephrine are the primary glucose counter regulatory factors that normally prevent or (more or less rapidly) correct hypoglycemia (Davis *et al.,* 2024). In insulin-deficient diabetes (exogenous) insulin levels do not decrease as glucose levels fall, and the combination of deficient glucagon and epinephrine responses causes defective glucose counter regulation (Demirbilek *et al.,* 2023). Furthermore, reduced sympathoadrenal responses can cause hypoglycemia unawareness (Hoelzen *et al.,* 2024). The concept of hypoglycemia-associated autonomic failure (HAAF) or Cryer syndrome in diabetes posits that recent incidents of hypoglycemia causes both defective glucose counter regulation and hypoglycemia unawareness (Sakar and Cinar, 2024). By shifting glycemic thresholds for the sympathoadrenal (including epinephrine) and the resulting neurogenic responses to lower plasma glucose concentrations, antecedent hypoglycemia leads to a vicious cycle of recurrent hypoglycemia and further impairment of glucose counter regulation (Hoelzen *et al.,* 2024). In many cases (but not all), short-term avoidance of hypoglycemia reverses hypoglycemia unawareness in affected patients, although this is easier in theory than in clinical experience (Hoelzen *et al.,* 2024). In most cases, hypoglycemia is treated with sugary drinks or food (Markus and Rogers, 2020). In severe cases, an injection of glucagon (a hormone with effects largely opposite to those of insulin) or an intravenous infusion of dextrose is used for treatment, but usually only if the person is unconscious (La Sala and Pontiroli, 2021). In any given incident, glucagon will only work once as it uses stored liver glycogen as a glucose source; in the absence of such stores, glucagon is largely ineffective

(Hoelzen *et al.,* 2024). In hospitals, intravenous dextrose is often used.

2.9.4 DIAGNOSIS OF TYPE 2 DIABETES MELLITUS

Type 2 diabetes is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency (James *et al.,* 2021). This is in contrast to type 1 diabetes in which there is an absolute insulin deficiency due to destruction of islet cells in the pancreas and gestational diabetes that is a new onset of high blood sugars associated with pregnancy (Kumar *et al.,* 2020). Type 1 and type 2 diabetes can typically be distinguished based on the presenting circumstances. If the diagnosis is in doubt antibody testing may be useful to confirm type 1 diabetes and C-peptide levels may be useful to confirm type 2 diabetes, with C-peptide levels normal or high in type 2 diabetes, but low in type 1 diabetes (Maddaloni *et al.,* 2022). The World Health Organization definition of diabetes (both type 1 and type 2) is for a single raised glucose reading with symptoms, or for raised glucose readings on two separate dates, of either (Kumar *et al.,* 2020).

i. FASTING PLASMA GLUCOSE

Measures your blood sugar after an overnight fast (at least 8 hours without food or drink). A level of 126 mg/dL or higher on two separate tests is diagnostic (Matthew and Tadi, 2020).

ii. ORAL GLUCOSE TOLERANCE TEST (OGTT)

Measures your blood sugar before and after you drink a glucose-rich beverage. A level of 200 mg/dL or higher two hours after drinking the beverage is diagnostic (Yameny, 2024).

iii. GLYCATED HEMOGLOBIN

Glycated hemoglobin, also called glycohemoglobin, is a form of hemoglobin (Hb) that is chemically linked to a sugar (Samanta, 2021). The formation of excess sugar-hemoglobin linkages indicates the presence of excessive sugar in the bloodstream and is an indicator of diabetes or other hormone diseases in high concentration (HbA1c > 6.4%) (Samanta, 2021). The process by which sugars attach to hemoglobin is called glycation and the reference system is based on HbAlc, defined as beta-N-1-deoxy fructosyl hemoglobin as a component (Ali, 2024). HbA1c is measured primarily to determine the three-month average blood sugar level and is used as a standard diagnostic test for evaluating the risk of complications of diabetes and as an assessment of glycemic control (Tao *et al*., 2023). The test is considered a three-month average because the average lifespan of a red blood cell is three to four months. Normal levels of glucose produce a normal amount of glycated hemoglobin (Hempe and Hsia, 2022).

2.9.5 MANAGEMENT OF TYPE 2 DIABETES MELLITUS

Management of type 2 diabetes focuses on lifestyle interventions, lowering other cardiovascular risk factors, and maintaining blood glucose levels in the normal range (Rosenfeld *et al.,* 2025). Self-monitoring of blood glucose for people with newly diagnosed type 2 diabetes may be used in combination with education, although the benefit of self-monitoring in those not using multi-dose insulin is questionable (Farrell and McCrimmon, 2021). In those who do not want to measure blood levels, measuring urine levels may be done. Managing other cardiovascular risk factors, such as hypertension, high cholesterol, and microalbuminuria, improves a person's life expectancy (Sharma *et al.,* 2020). Decreasing the systolic blood pressure to less than 140 mmHg is associated with a lower risk of death and better outcomes (Fuchs and Whelton, 2020). Intensive blood pressure management (less than 130/80 mmHg) as opposed to standard blood pressure management (less than 140-160 mmHg systolic to 85-100 mmHg diastolic) results in a slight decrease in stroke risk but no effect on overall risk of death (Fuchs and Whelton, 2020). Intensive blood sugar lowering (HbA < 6%) as opposed to standard blood sugar lowering (HbAc of 7-7.9%) does not appear to change mortality. The goal of treatment is typically an HbAn Of 7 to 8% or a tasting glucose of less than 7.2 mmol/L (130 mg/dL); however, these goals may be changed after professional clinical consultation, taking into account particular risks of hypoglycemia and life expectancy (Fuchs and Whelton, 2020). Hypoglycemia is associated with adverse outcomes in older people with type 2 diabetes (Mattishent and Loke, 2021). Despite guidelines recommending that intensive blood sugar control be based on balancing immediate harms with long-term benefits, many people for example people with a life expectancy of less than nine years who will not benefit, are over-treated (Sacks *et al.,* 2023). It is recommended that all people with type 2 diabetes get regular eye examination. There is moderate evidence suggesting that treating gum disease by scaling and root planning results in an improvement in blood sugar levels for people with diabetes (Bagde *et al.,* 2023).

i. EXERCISE

A proper diet and regular exercise are foundations of diabetic care, with one review indicating that a greater amount of exercise improved outcomes (Magkos *et al.,* 2020). Regular exercise may improve blood sugar control, decrease body fat content, and decrease blood lipid levels (Karami *et al.,* 2021).

ii. DIET

Calorie restriction to promote weight loss is generally recommended (Magkos, 2022). Around 80 percent of obese people with type 2 diabetes achieve complete remission with no need for medication if they sustain a weight loss of at least 15 kilograms (33 b), but most patients are not able to achieve or sustain significant weight loss (Magkos, 2022). Even modest weight loss can produce significant improvements in glycemic control and reduce the need for medication (Lazzaroni *et al.,* 2021). Several diets may be effective such as the DASH diet, Mediterranean diet, low-fat diet, or monitored carbohydrate diets such as a low carbohydrate diet. Other recommendations include emphasizing intake of fruits, vegetables, reduced saturated fat and low-fat dairy products, and with a macronutrient intake tailored to the individual, to distribute calories and carbohydrates throughout the day (Lazzaroni *et al.,* 2021). A 2021 review showed that consumption of tree nuts (walnuts, almonds, and hazelnuts) reduced fasting blood glucose in diabetic people (Muley *et al.,* 2021). As of 2015, there is insufficient data to recommend nonnutritive sweeteners, which may help reduce caloric intake (Ashwell *et al.,* 2020). An elevated intake of microbiota-accessible carbohydrates can help reducing the effects of T2D (Xu *et al.,* 2021). Viscous fiber supplements may be useful in those with diabetes. Culturally appropriate education may help people with type 2 diabetes control their blood sugar levels for up to 24 months (Sampath *et al.,* 2025). There is not enough evidence to determine if lifestyle interventions affect mortality in those who already have type 2 diabetes.

iii. STRESS MANAGEMENT

Although psychological stress is recognized as a risk factor for type 2 diabetes, the effect of stress management interventions on disease progression are not established. A Cochrane review is under way to assess the effects of mindfulness-based interventions for adults with type 2 diabetes.

iv. BLOOD PRESSURE LOWERING

Many international guidelines recommend blood pressure treatment targets that are lower than 140/90 mmHg for people with diabetes (Kim and Kim, 2022). However, there is only limited evidence regarding what the lower targets should be. A 2016 systematic review found potential harm to treating to targets lower than 140 mmHg, and a subsequent review in 2019 found no evidence of additional benefit from blood pressure lowering to between 130 and 140 mmHg, although there was an increased risk of adverse events (Saiz *et al.,* 2022). 2023 European Society of Cardiology guidelines recommend systolic blood pressure lowering to 130 mmHg in most people with diabetes (Kjeldsen *et al.,* 2025). In people with diabetes and hypertension and either albuminuria or chronic kidney disease, an inhibitor of the renin-angiotensin system (such as an ACE inhibitor or angiotensin receptor blocker) to reduce the risks of progression of kidney disease and present cardiovascular events (Bhandari *et al.,* 2022). There is some evidence that angiotensin converting enzyme inhibitors (ACEls) are superior to other inhibitors of the renin-angiotensin system such as angiotensin receptor blockers (ARBs), 1oraliskiren in preventing cardiovascular disease (Ahmad *et al.,* 2023). Although a 2016 review found similar effects of ACEls and ARBs on major cardiovascular and renal outcomes (Saiz *et al.,* 2022). There is no evidence that combining ACEls and ARBs provides additional benefits.

2.10 GESTATIONAL DIABETES MELLITUS

Gestational diabetes is a condition in which a woman without diabetes develops high blood sugar levels during pregnancy (Simmons *et al.,* 2023). Gestational diabetes generally results in few symptoms. Obesity increases the rate of pre-eclampsia, cesarean sections, and embryo macrosomia, as well as gestational diabetes (Simmons *et al.,* 2023). Babies born to individuals with poorly treated gestational diabetes are at increased risk of macrosomia, of having hypoglycemia after birth, and of jaundice (Bernea *et al.,* 2022). If untreated, diabetes can also result in stillbirth (Lemieux *et al.,* 2022). Long term, children are at higher risk of being overweight and of developing type 2 diabetes (Lemieux *et al.,* 2022). Gestational diabetes can occur during pregnancy because of insulin resistance or reduced production of insulin (Simmons *et al.,* 2023). Risk factors include being overweight, previously having gestational diabetes, a family history of type 2 diabetes, and having polycystic ovarian syndrome (Mills *et al.,* 2020). Diagnosis is by blood tests. For those at normal risk, screening is recommended between 24 and 28 weeks' gestation. For those at high risk, testing may occur at the first prenatal visit. Maintenance of a healthy weight and exercising before pregnancy assist in prevention (Mills *et al.,* 2020). Gestational diabetes is treated with a diabetic diet, exercise, medication (such as metformin), and sometimes insulin injections (Mukherjee and Dawson, 2022). Most people manage blood sugar with diet and exercise (Guha *et al.,* 2020). Blood sugar testing among those affected is often recommended four times daily (Guha *et al.,* 2020). Breastfeeding is recommended as soon as possible after birth. Gestational diabetes affects 3-9% of pregnancies, depending on the population studied (Ye *et al.,* 2022). It is especially common during the third trimester. It affects 1% of those under the age of 20 and 13% of those over the age of 44 (Ye *et al.,* 2022). Several ethnic groups including Asians, American Indians, Indigenous Australians, and Pacific Islanders are at higher risk. criteria. In 90% of cases, gestational diabetes resolves after the baby is born (Simmons *et al.,* 2023). Affected people, however, are at an increased risk of developing type 2 diabetes.

2.11 MEDICINAL PLANTS USED TO TREAT DIABETES MELLITUS

i. *Arctium lappa*

Arctium lappa, commonly called greater burdock is used traditionally as a diuretic and to lower blood sugar and, in traditional Chinese medicine as a treatment for sore throat and symptoms of the common cold (Mir *et al.,* 2022).

ii. *Allium sativium*

Allium sativum is the scientific name for garlic, which is a plant species in the family of Amaryllidaceae (Sasi *et al.,* 2021). Garlic is known to have various health benefits such as lowering cholesterol, improving blood pressure, and boosting the immune system (Ansary *et al.,* 2020). Garlic has also been shown to have potential benefits for individuals with diabetes. Research has suggested that garlic may help lower blood sugar levels and improve insulin sensitivity, which can be beneficial for diabetes (Dereje *et al.,* 2025).

iii. *Ocium gratissimum*

Ocimum gratissimum, commonly known as scent leaf, exhibits anti-diabetic properties, primarily through its ability to lower blood glucose levels (Ugbogu *et al.,* 2021). Studies suggest this effect is achieved by inhibiting alpha-glucosidase, an enzyme involved in carbohydrate digestion, thereby reducing post-meal glucose spikes (Bhatnagar and Mishra, 2022). Additionally, various leaf extracts of *O. gratissimum* have shown potential in managing type 2 diabetes by enhancing glucose tolerance and reducing fasting blood glucose levels in animal models (Shittu *et al.,* 2021).

2.12 PANCREATIC LIPASES

Pancreatic lipases are a family of lipolytic enzymes that hydrolyses ester linkages of triglycerides (Lim *et al.,* 2022). Lipases are widely distributed in animals, plants and prokaryotes (Chandra *et al.,* 2020). At least three tissue-specific isozymes exist in higher vertebrates, pancreatic, hepatic and gastric/lingual (Chandra *et al.,* 2020). These lipases are closely related to each other and to lipoprotein lipase, which hydrolyses triglycerides of chylomicrons and very low density lipoproteins (VLDL). Pancreatic lipase contains two protein domains (Salhi *et al.,* 2021). The one toward the N terminus is an aß hydrolase, whereas the one toward the C terminus plays a role in binding to colipase, a protein needed in order for the lipase to become activated (Salhi *et al.,* 2021). The most conserved region in all these proteins is centered on a serine residue which has been shown to participate, with a histidine and an aspartic acid residue, in a charge relay system. Such a region is also present in lipases of prokaryotic origin and in lecithin-cholesterol acyitransferase (LCAT), which catalyzes fatty acid transfer between phosphatidylcholine and cholesterol.

2.13 GAS CHROMATOGRAPHY: MASS SPECTROMETRY

Gas chromatography-mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample (Maji and Roy, 2023). Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, food and flavor analysis, and identification of unknown samples, including that of material samples obtained from planet Mars during probe missions as early as the 1970s (Bubli *et al.,* 2021). GC-MS can also be used in airport security to detect substances in luggage or on human beings (Maji and Roy, 2023). Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification. Like liquid chromatography-mass spectrometry, it allows analysis and detection even of tiny amounts of a substance (Thomas *et al.,* 2022). GC-MS has been regarded as a "gold standard" for forensic substance identification because it is used to perform a 100% specific test, which positively identifies the presence of a particular substance (Gould *et al.,* 2023). A nonspecific test merely indicates that any of several in a category of substances is present. Although a nonspecific test could statistically suggest the identity of the substance, this could lead to false positive identification (Gould *et al.,* 2023). However, the high temperatures (300°C) used in the GC-MS injection port (and oven) can result in thermal degradation of injected molecules, thus resulting in the measurement of degradation products instead of the actual molecule(s) of interest (Campbell *et al.,* 2020).

CHAPTER THREE

3.0 METHODOLOGY

3.1 CHEMICALS AND REAGENTS

The reagents and chemicals used in biochemical assays carried out includes:

- Folin-Ciocalteu Reagent (FCR) (Pyrex, Nigeria)
- Gallic acid (Pyrex, Nigeria)
- Ascorbic acid (Sigma, United Kingdom)
- Aluminum Chloride ($ALCl_3$) (Pyrex, Nigeria)
- Quercetin (Pyrex, Nigeria)
- Methanol (Pyrex, Nigeria)
- Sodium Nitrite (NaNO) (Pyrex, Nigeria)
- Sulphuric Acid (H_2SO_4) (Pyrex, Nigeria)
- Ethanol (Pyrex, Nigeria)
- Sodium Hydroxide (NaOH) (Pyrex, Nigeria).

All reagents were of analytical grade and distilled water was used for all biochemical assays.

3.2 EQUIPMENT

The equipment used in biochemical assays carried out includes:

- Sensitive Electronic Weighing Balance (TYPE LAC241C, 704010).
- U-V Spectrophotometer (Searchtech Instruments, United Kingdom, Model: AE-560-20)
- Hisense Refrigerator (Model: HTF-319H)
- Thermostat Oven (Model: DHG-9053A)
- Rotators Evaporator (Model: RE-201D)
- Haier Thermocool Chest Freezer (Model: HTF-319H)
- Water Bath (Searchtech instruments, United Kingdom, Model: HH-S6)
- Freeze Dryer (LGJ-10-80C United Kingdom)
- Water Distiller

3.3 PLANT COLLECTION

The leaf of *Ocimum gratissimum* was obtained from Ovia North East Local Government Area of Edo State, University of Benin, Benin City, Nigeria. After which it was properly identified at the herbarium unit of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State. Subsequently, it underwent identification at the Department of Microbiology and Botany at the University of Benin, Nigeria and a voucher number was given for the plant as UBH-0333.

3.4 PLANT EXTRACTION

Fresh leaves of *Ocimum gratissimum* were first plucked out from the stems, and the leaves were thoroughly washed under clean running tap water in the laboratory to remove adhering dirt and debris, and air-dried under shade at room temperature with adequate ventilation to prevent microbial growth and degradation of phytochemicals. Drying continued until the leaves became crisp and brittle, after which they were pulverized into a coarse powder using a clean, dry blender and were then weighed using an electronic sensitive weighing balance. The total weight of the sample was 80g. The powdered sample was stored in airtight containers until further use. Extraction of the powdered leaves was performed by exhaustive maceration using distilled water as solvent. 80g of the leaf powder was soaked in distilled water in a transparent bowl, tightly covered and left to stand at room temperature for 72 hours. During the period of maceration, the mixture was stirred intermittently (2–3 times daily) to enhance solvent penetration and solubilization of phytochemicals. After soaking, the mixture was filtered using a double layered cheese cloth as a medium membrane. The residual plant material (marc) obtained after the first extraction was re-soaked in fresh distilled water of equal volume and extracted again for 48 and 24 hours respectively under the same conditions with intermittent stirring. Afterward, the mixture was filtered through the double layered cheese cloth as previously described, and the filtrate collected. The pooled extracts from both cycles were then subjected to clarification using thick cotton wool filtration. The crude aqueous extract was passed repeatedly through a tightly packed layer of thick cotton wool until a clearer filtrate free of fine debris was obtained. The extraction process was done 5 times to obtain exhaustive extraction. This step ensured removal of mucilage and suspended particles that could interfere with subsequent analysis. At the end of the extraction, the extract was taken to the TRIGAS research laboratory for freeze drying using a laboratory lyophilizer. A weight of 9g was obtained after freeze drying. This was kept in an airtight transparent container in a freezer until required for GC–MS profiling and *in-vitro* anti-pancreatic lipase studies.

3.5 BIOCHEMICAL ASSAYS

The anti-pancreatic lipase activities of the *in vitro* leaf extract of *Ocimum gratissimum* were evaluated using the following biochemical assays:

3.6 GC-MS ANALYSIS PROCEDURE

The extracted sample was analyzed using SHIMADZU GC-2010, equipped with OPTIMA XLB column (60 m X 0.25 mm i.d., 0.25 um). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used Helium was used as the carrier gas at a flow rate of 1 mL/min. Injector and MS transfer line temperatures was set at 220°C and 290°C, respectively. The program was used at 50-150°C at a rate of 3°C/min. Diluted samples was injected in split mode. The components were identified based on the NIST library data of the GC-MS system and literature data (Adams, 2001).

3.7 PROTOCOL FOR BIOINFORMATIC TECHNIQUE

i. Ligand preparation

The 3-Dimensional (3D) structures of 27 phytocompounds from the GC-MS analysis of the aqueous leaf extract of *Ocimum gratissimum* and a reference drug (Orlistat) were validated and retrieved from PubChem in 3D SDF file format. The OpenBabel interface built in the PYRX docking tool was utilized at default settings to optimise the ligands for docking by minimizing their energy level and were converted to Autodock ligand Pdbqt files in preparation for docking.

ii. Protein preparation

The 3D crystal structures of pancreatic lipase with PDB ID (IN8S) were retrieved from the Protein Data Bank in the PDB file format. The enzyme was repaired for docking by removing the native ligands, unbound water molecules, and previously bound ligands, while polar hydrogen atoms and charges were added using UCSF Chimera software. CastP, an online web server, was used for the active site prediction of the enzyme and structure. The structures were further optimized to Autodock macromolecule and converted to Pdbqt form using the PYRX software before docking.

iii. Molecular Docking

Molecular docking is an essential computational chemistry approach in rational drug design that could assist in drug production to treat a broad range of disorders and in treatment design for disease prevention. This approach is a convenient and cheap means to study protein-ligand interactions. Binding energy is the major factor produced as a molecular docking outcome. The hint of strength and affinity of the interaction between the ligand and the receptor is provided by the binding energy. The lower the binding energy is, the stronger the interaction. Negative binding energy designates the best binding affinity of the ligand with the receptor molecule which is an essential property of a good drug. Autodock Vina interface on PYRX docking tool was used to perform the molecular docking study. The protein-ligand interactions were predicted by docking the phytocompounds into the active pockets of the targets with PYRX docking software. The active pockets on the target proteins were set to be at the middle of the grid box and the docking was performed at default setting. Macromolecule-ligand complexes were formed using Notepad++. The visualization of the binding poses in 2 and 3-Dimensional structures were performed with BIOVIA discovery studio software.

1. ***In silico* ADMET prediction**

In silico methods for the determination of Absorption-Distribution-Metabolism-Excretion (ADME) parameter is based on theoretically generated statistical models, which have been generated by comparing the structural elements of compounds that have been obtained in a given assay to their biological responses (Gleeson *et al.,* 2011). ADME study was further used to screen the phytocompounds. The pharmacokinetic and pharmacodynamics factors such as blood brain barrier penetration, human gastrointestinal absorption, cytochrome P450 solubility, drug-likeliness and the effector site prediction for these phytocompounds was carried out by submitting their canonical SMILES to swissADME webserver (http://www.swissadme.ch).

3.8 STATISTICAL ANALYSIS

The data were expressed as means of 4 to 7 determinations ± S.E.M. The differences among groups were analyzed by the one-way analysis of variance (ANOVA). Inter-group comparisons were done by Duncan's post hoc test. A value of $P < 0.05$ was accepted as significant. Graphical Prism version 8.0.1 was used for this analysis and plotting of the graph.

CHAPTER FOUR

4.0 RESULTS

4.1 GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS) ANALYSIS OF THE AQUEOUS LEAF EXTRACT OF *Ocimum gratissimum*

The GC-MS analysis of the aqueous leaf extract of *Ocimum gratissimum* revealed 27 distinct phytochemical constituents with varying retention times, peak heights, and relative peak areas (Table 4.1). Among the identified compounds, Supraene (17.71%), Glycerin (11.38%), 1,4-Dimethoxy-2,3-dimethylbenzene (8.07%), Acetamide, N-butyl (7.11%), and Thymol (5.86%) were the predominant components based on their peak areas. These high proportions indicate that they are the most abundant metabolites found in the extract.

Table 4.1: GC–MS Identified Phytocompounds in the Aqueous Leaf Extract of *Ocimum gratissimum*

Phytocompounds	Retention Time (min)	Height (%)	Area (%)
Cyclohexanone	9.594	2.65	3.05
Glycerin	10.542	4.56	11.38
4-Heptanone, 3-methyl	11.357	1.4	1.57
Cyclopropyl carbinol	12.28	1.65	1.3
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6	12.845	2.48	2.14
trans-2-Undecen-1-ol	13.05	2.12	1.51
Catechol	13.432	3.61	4.87
Benzofuran, 2,3-dihydro	13.685	1.21	0.97
Thymol	14.479	6.89	5.86
1,4-dihydroxy-p-menth-2-ene	14.737	3.58	2.27
1,3-Dioxolane, 2,2-dimethyl4,5-bis(1-methyle	14.809	3.27	1.97
2-Methoxy-4-vinylphenol	14.991	2.61	1.78
1,3-Dioxolane, 2,2-dimethyl-4,5-bis(1-methylethenyl)	15.04	2.85	1.69
2,7-0Octadiene-1,6-diol, 2,6-dimethyl	15.381	1.03	0.96
Acetamide, N-butyl	15.578	5.62	7.11
Benzenemethanol, α,α,4-trimethyl	16.291	1.39	1.07
2-Hydroxy-5-methylisophthalaldehyde	16.692	1.55	1.46
Benzenemethanol, 4-(methoxymethyl)-α-methyl	16.972	5.99	4.85
5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	17.31	1.33	1.1
t-Butylhydroquinone	17.833	1.31	1.03
1,4-Dimethoxy-2,3-dimethylbenzene	18.055	10.93	8.07
Supraene	19.179	16.42	17.71
2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl	20.761	2.04	1.66
7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)	20.981	1.28	1.05
2,6,6-Trimethyl-3-(phenylthio)cyclohept-4-enol	21.581	1.57	1.67
2-methyloctacosane	23.489	0.91	2.03

4.2 MOLECULAR DOCKING ANALYSIS

The molecular docking analysis from the study reveals the binding affinity of the phytocompounds and standard drug (Orlistat) with the protein (pancreatic lipase). The binding affinity of the phytocompounds ranged from -3.5 to -7.1 kcal/mol against the pancreatic lipase receptor. From the study, it was observed that the strongest interactions were recorded for β-Selinene (–7.1 kcal/mol), α-Selinene (–6.5 kcal/mol), Benzenemethanol, alpha.,alpha.,4-trimethyl(-6.2kcal/mol), 2,6,6-Trimethyl-3-(phenylthio)cyclohept-4-enol (–6.1 kcal/mol), 2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl) (-5.9 kcal/mol), 1,4-dihydroxy-p-menth-2-ene (-5.7 kcal/mol), t-Butylhydroquinone (-5.7 kcal), Thymol (-5.7 kcal/mol), 1,3-Dioxolane, 2,2-dimethyl-4,5-bis(1-methylethenyl) (-5.6 kcal/mol), 5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol (-5.6 kcal/mol), Benzenemethanol, 4-(methoxymethyl)-alpha.-methyl (-5.6 kcal/mol), 7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl (-5.5 kcal/mol), Catechol (-5.5 kcal/mol), 2-Hydroxy-5-methylisophthalaldehyde (-5.4 kcal/mol), 2-Methoxy-4-vinylpheno (-5.4 kcal/mol), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methy (-5.4 kcal/mol), Supraene (-5.4 kcal/mol), Benzofuran, 2,3-dihydro (-5.3 kcal/mol), all of which demonstrated stronger binding affinities than the standard pancreatic lipase drug (Orlistat) which had a binding score of -5.2 kcal/mol. The 2D and 3D interactions of the selected compounds (α-Selinene, β-Selinene) and the standard drug (Orlistat), further illustrate their binding poses within the pancreatic lipase binding active site, as shown in Figures 4.2.1 to 4.2.3

Table 4.2: Docking Results (kcal/mol) of 27 Phytocompounds from *Ocimum gratissimum*

Phytocompounds	PUBCHEM ID	Binding affinity (Kcal/mol)
1,3-Dioxolane, 2,2-dimethyl-4,5-bis(1-methylethenyl)	539045	-5.6
1,4-dihydroxy-p-menth-2-ene	300085	-5.7
1,4-Dimethoxy-2,3-dimethylbenzene	148299	-5
2,6,6-Trimethyl-3-(phenylthio)cyclohept-4-enol	585064	-6.1
2,7-Octadiene-1,6-diol, 2,6-dimethyl	5280678	-5.2
2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)	5280662	-5.9
2-Hydroxy-5-methylisophthalaldehyde	81744	-5.4
2-Methoxy-4-vinylpheno	332	-5.4
2-methyloctacosane	519147	-4.8
4-Heptanone, 3-methyl	27470	-4.3
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methy	119838	-5.4
5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	565280	-5.6
7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl	232703	-5.5
Acetamide, N-butyl	61265	-4.1
Alpha-Selinene	10856614	-6.5
Benzenemethanol, 4-(methoxymethyl)-alpha.-methyl	591895	-5.6
Benzenemethanol, alpha.,alpha.,4-trimethyl	601306	-6.2
Benzofuran, 2,3-dihydro	10329	-5.3
Beta-Selinene	442393	-7.1
Catechol	289	-5.5
Cyclohexanone	7967	-4.4
Cyclopropyl carbinol	75644	-3.5
Glycerin	753	-4.3
Orlistat	3034010	-5.2
Supraene	638072	-5.4
t-Butylhydroquinone	16043	-5.7
Thymol	6989	-5.7
trans-2-Undecen-1-ol	5365004	-4.4

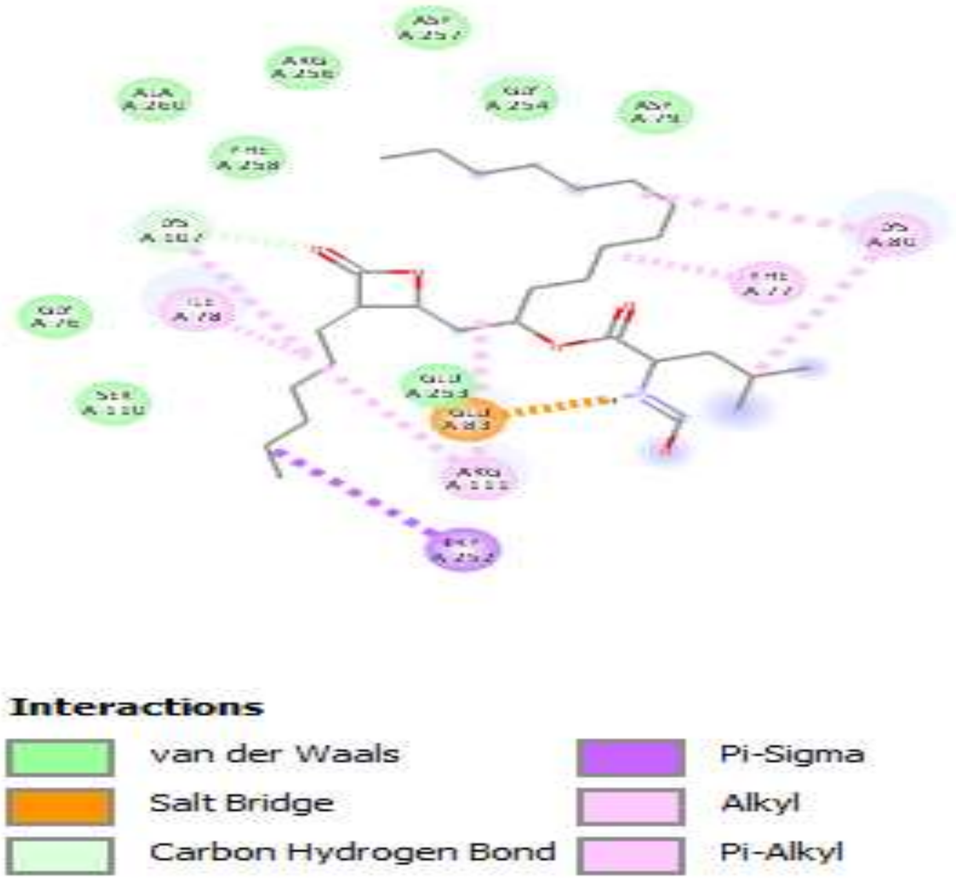

Figure 4.2.1a: 2D Ligands interaction of Orlistat with pancreatic lipase.

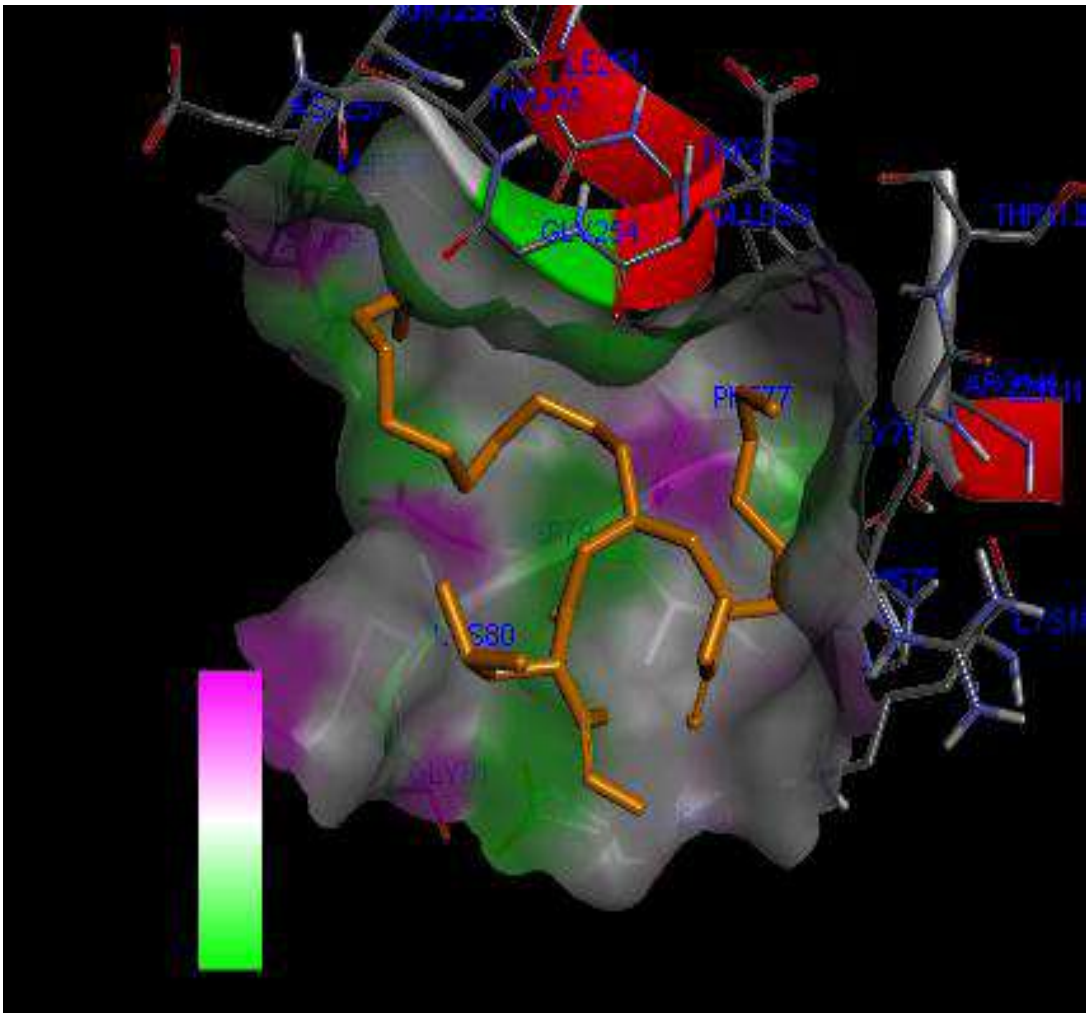

Figure 4.2.1b: The binding pose of Orlistat with pancreatic lipase.

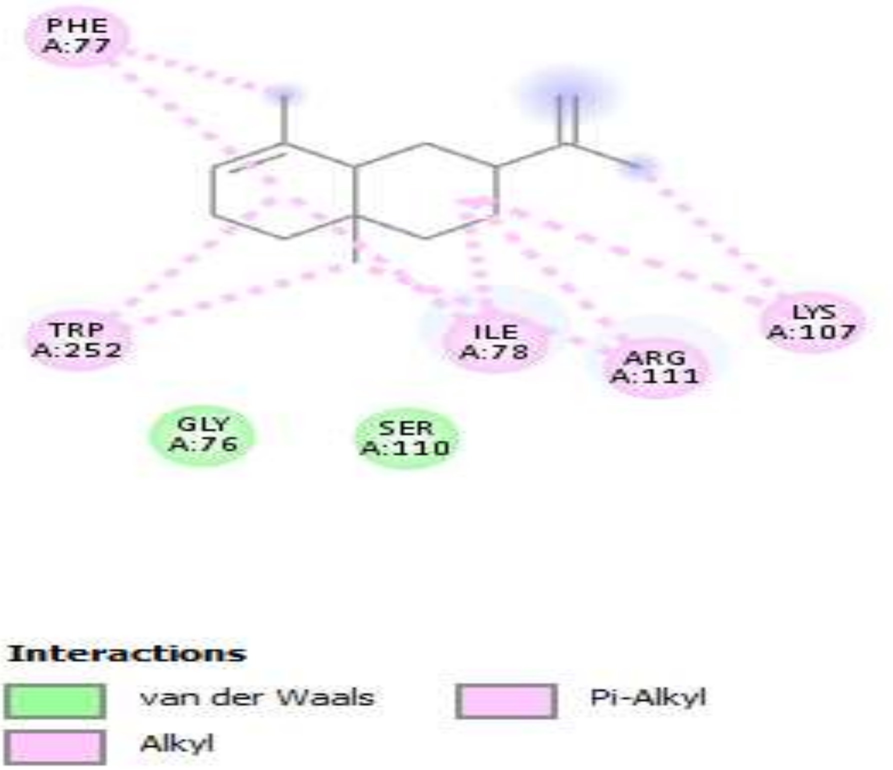

Figure 4.2.2a: The 2D Ligand interaction of α-Selinene with pancreatic lipase.

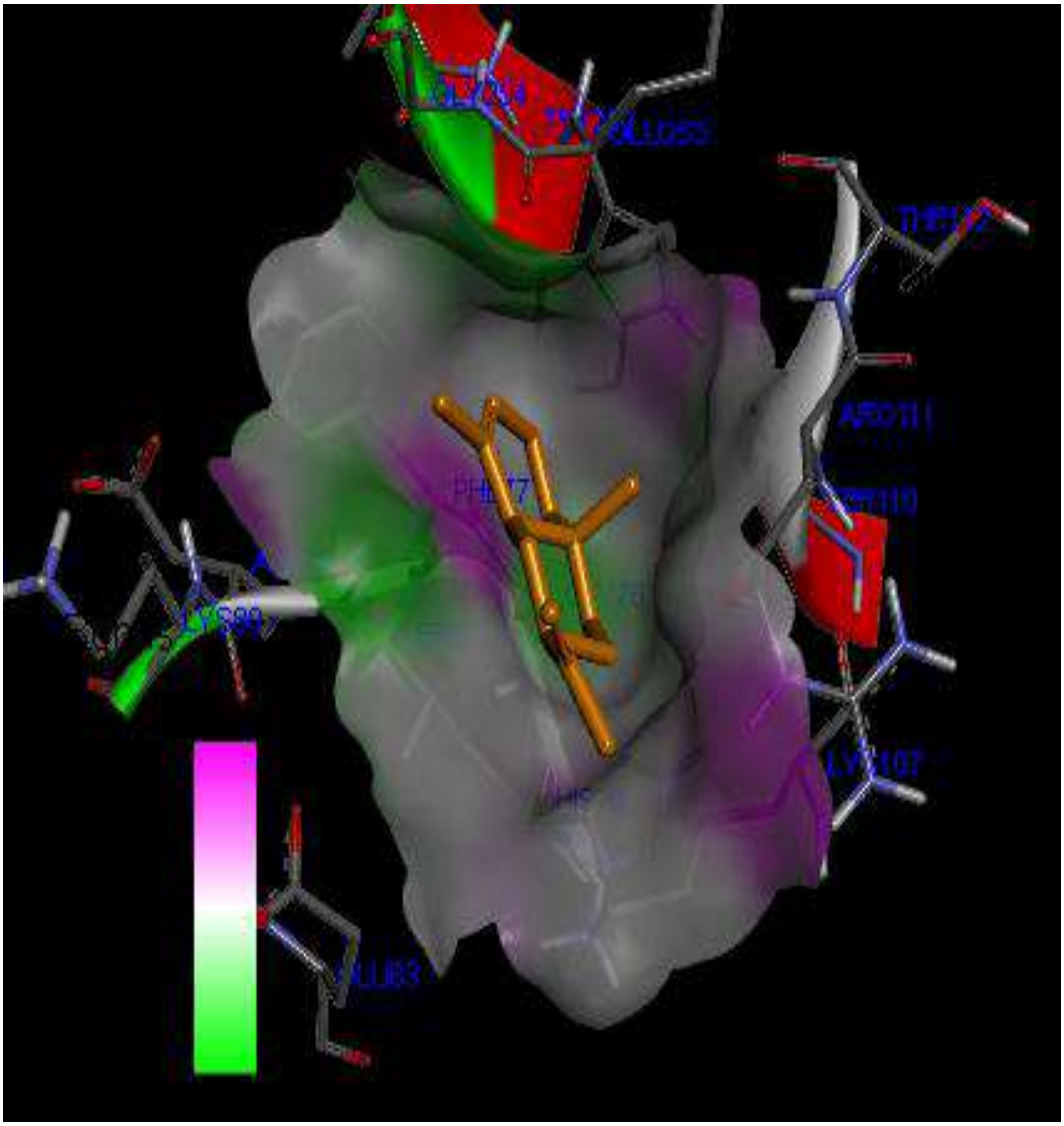

Figure 4.2.2b: The binding pose of α-Selinene at the binding pocket of pancreatic lipase.

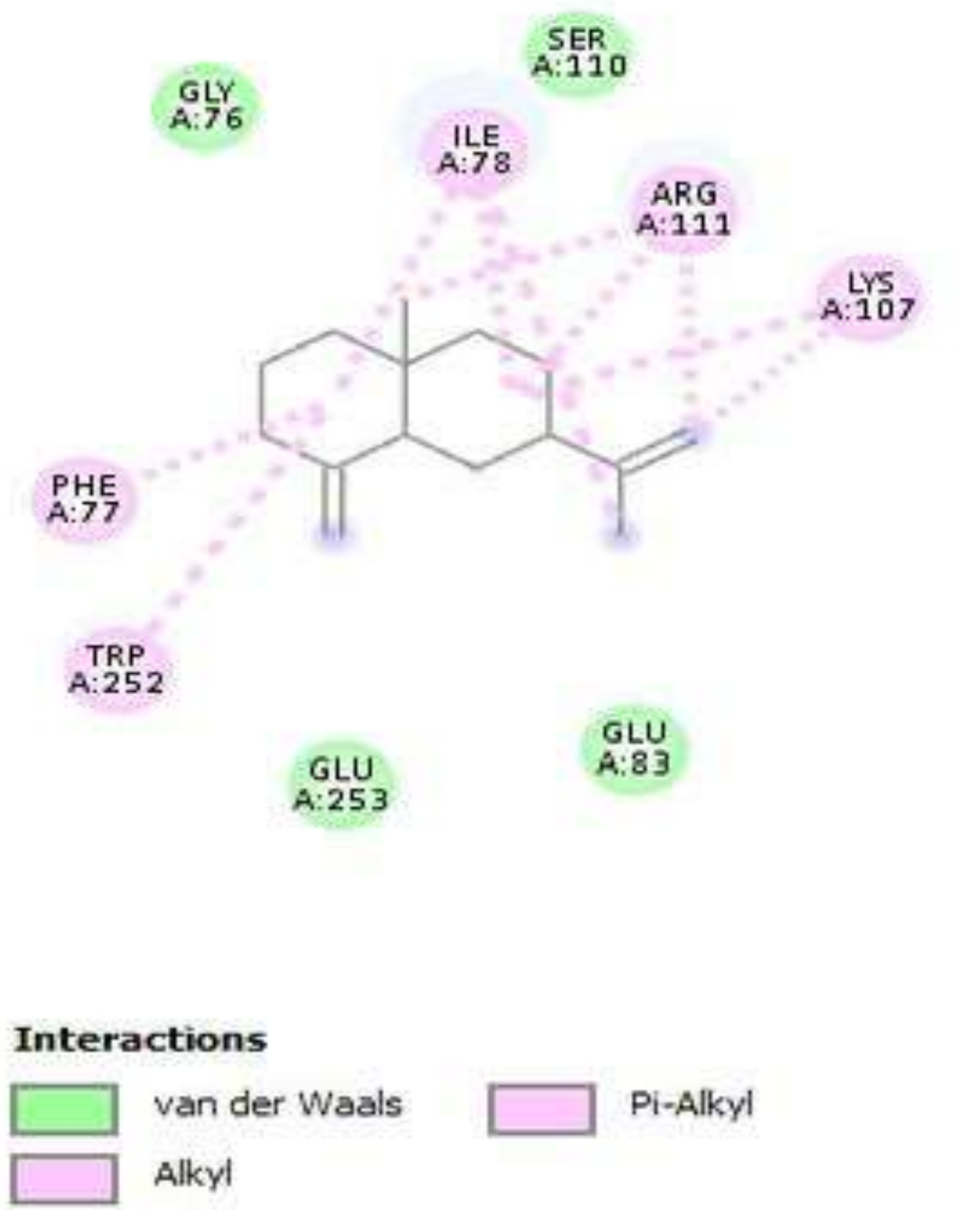

Figure 4.2.3a: The 2D Ligand interaction of β-Selinene with pancreatic lipase.

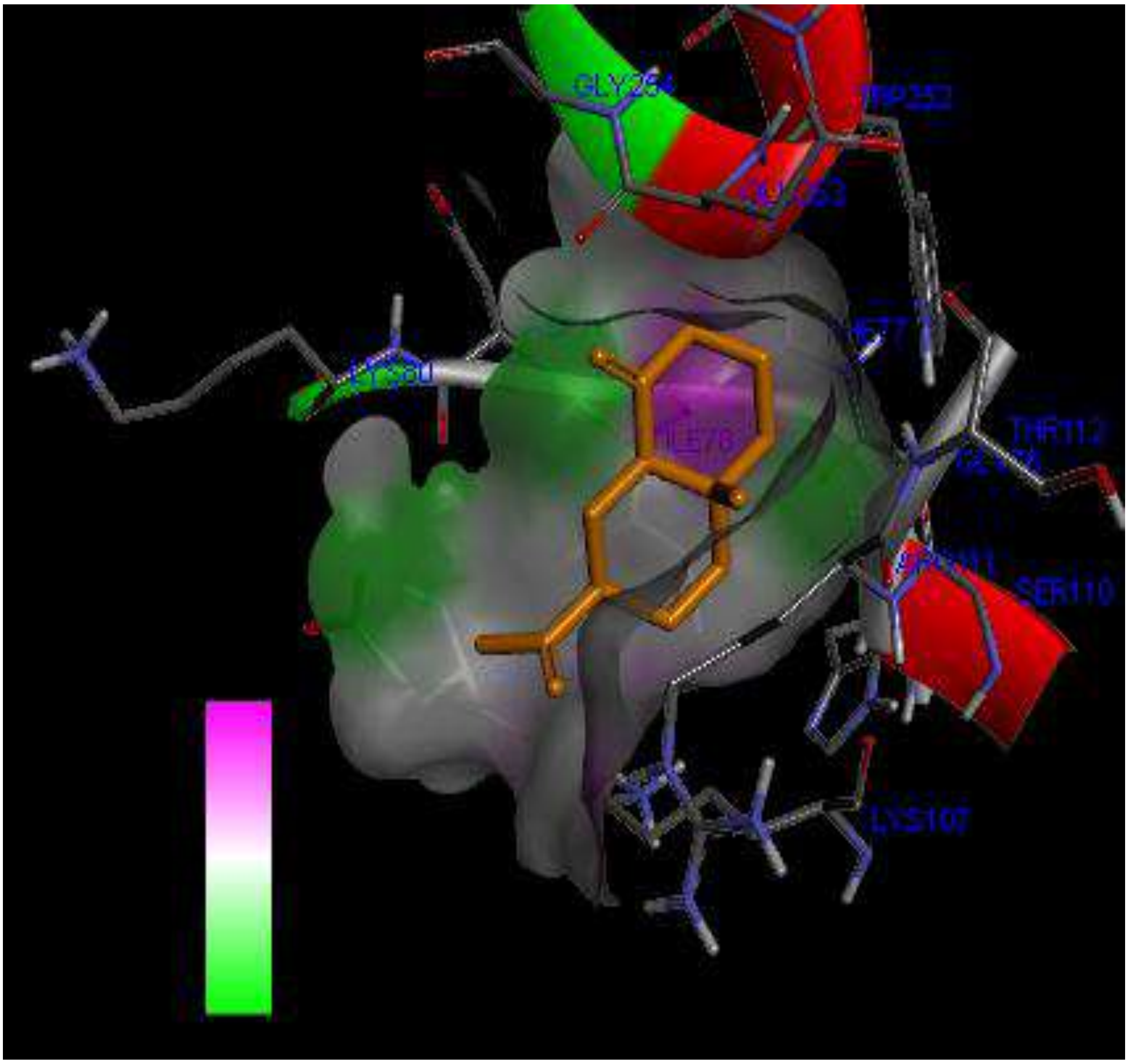

Figure 4.2.3a: The binding pose of β-Selinene at the binding pocket of pancreatic lipase.

CHAPTER FIVE

5.0 DISCUSSION

Diabetes mellitus represents a perpetual metabolic ailment demonstrating ongoing elevated blood sugar levels for patients who show either secretory pancreatic failure or cellular response failure to insulin or both (Sarkar *et al.,* 2019). Patients with diabetes show disrupted metabolism of carbohydrates alongside fats and proteins which results into long-term organ injuries to both eyes and kidneys and nerves and blood vessels (Awuchi, *et al.,* 2020). Autoimmune destruction of pancreatic β-cells results in type 1 diabetes leading to absolute lack of insulin that requires continuous insulin treatment (Roep *et al*., 2021). The most frequently occurring diabetes form called type 2 diabetes develops when patients develop reduced cellular sensitivity to insulin together with gradually less insulin production which depends heavily on both lifestyle patterns and genetic traits (Pillon *et al*., 2021). Gestational diabetes emerges through hormonal-driven insulin resistance during pregnancy and holds significant potential to cause Type 2 diabetes in the future (Simmons *et al.,* 2023). Diabetes mellitus stands as a fundamental worldwide medical priority which demands prompt detection along with continuous therapeutic care. The management of diabetes traditionally involves lifestyle modification, oral anti-diabetic drugs, insulin therapy, and in some cases, combination treatments (Tegegne *et al*., 2024). However, these therapies mainly target blood glucose regulation and do not fully address the downstream biochemical events such as anti-pancreatic lipase. As a result, medicinal plants containing antioxidant and anti-pancreatic lipase phytochemicals are increasingly explored as safer and more affordable alternatives or complementary therapies for diabetes management (Ugbogu *et al*., 2021).

Ocimum gratissimum (Scent leaf), represents a common medicinal herb because of both its abundant phytochemical constituents and extensive therapeutic potentials (Ojewumi *et al*., 2024). Its traditional medicinal uses include control of infections alongside treatment of inflammatory conditions and gastrointestinal complaints due to potent anti-microbial, anti-inflammatory, and anti-diabetic properties (Ugbogu *et al*., 2021). In this study, GC-MS analysis and molecular docking were employed to evaluate the phytochemical constituents of its aqueous leaf extract and to determine their potential anti-pancreatic lipase effects using *in silico* technique.

From the study, the *in-vitro* GC–MS analysis revealed 27 bioactive compounds, of which supraene was found to be the most abundant in the extract (17.71%). Supraene is a bioactive terpenoid-like compound proposed to possess promising anti-diabetic properties through its action on a wide range of metabolic pathways (Ridho *et al*., 2023). Supraene potentiates insulin signaling and promotes glucose uptake in peripheral tissues in support of better glycemic control. Besides these actions, supraene exerts anti-inflammatory effects that protect pancreatic tissue against cytokine-induced damage (Abduh *et al*., 2024). Glycerin, another compound identified from this study (11.38%), is known to serve as a non-glucose energy source and does not raise blood sugar significantly, thus being safe for diabetic patients as a sweetener (Alamgir, 2018). It undergoes slow metabolism in the liver into glucose through gluconeogenesis without causing much post-prandial glycemic impact. Its humectant effect helps to maintain skin hydration, which is useful in preventing diabetic complications of skin dryness, cracking, and poor wound healing. Though not an anti-diabetic drug by itself, its low glycemic index, metabolic stability, and dermatologic benefits make glycerin a useful adjunctive agent in the management of diabetes (Sharif *et al*., 2020). 1,4-Dimethoxy-2,3-dimethylbenzene (8.07%), also found in this study, has been shown from literature to possess antioxidant and anti-inflammatory activities that reduce oxidative stress which leads to insulin resistance and β-cell damage typical of Type 2 diabetes (Unuofin *et al.,* 2020). Research evidence indicates this substance can increase insulin-mediated glucose assimilation in body tissues by activating insulin response processes and altering cell-signaling mechanisms (Unuofin *et al.,* 2020). The compound acts as an antioxidant by defending pancreatic β-cells against free-radical damage enabling insulin secretion to remain stable. As an anti-inflammatory agent it helps decrease the kind of inflammation which persists for long periods and which leads to diabetic complications (Sadeghi *et al*., 2024). N-Butyl acetamide another compound identified (7.11%) from this study, is attributed to its health benefits through antioxidant properties as well as anti-inflammatory activities which both work against oxidative stress which causes insulin resistance together with damage to pancreatic β-cells (Anastasiou *et al*., 2021). Research teams through experimental assays demonstrate that N-Butyl acetamide enhances insulin sensitivity along with increasing cellular glucose uptake to improve glucose tolerance (Anastasiou *et al*., 2021). The compound demonstrates protective effects on pancreatic β-cells while decreasing hyperglycemia-related toxicity in pancreatic tissue (Unuofin *et al*., 2020). Thymol (5.86%)

known for its antioxidant capability counteracts free-radical damage to pancreatic β-cells leading to both better insulin secretion and cell preservation (Gandhi *et al*., 2024), was also identified in this study using GC-MS. Thymol demonstrates powerful anti-inflammatory capabilities by preventing or reducing the prolonged inflammation which obstructs proper insulin function (Gago *et al*., 2025). Studies demonstrate that thymol strengthens glucose absorption into body tissues through bettered insulin performance and enzyme operation in the carbohydrate metabolic pathway (Gago *et al*., 2025). The presence of these bioactive agents shows the therapeutic importance of the aqueous leaf extract of *O. gratissimum*. The complexity of the GC–MS profile supports the idea that *O. gratissimum* exerts its medicinal effects through synergistic interactions, rather than a single active compound (Ugbogu *et al*., 2021).

The predicted docking score for the phytocompounds from the aqueous leaf extract of *O. gratissimum* with the reference drug (Orlistat) against the targets in this study, were presented in the form of binding energy (kcal/mol). The binding interactions of the 27 phytocompounds identified in this study as well as the standard (Orlistat), were docked against pancreatic lipase. Orlistat, the reference drug docked against pancreatic lipase had a docking score of -5.2 kcal/mol which surpasses the docked score of some of the screened phytocompounds. While most of the phytocompounds had better binding scores than the standard, alpha-selinene (-6.5 kcal/mol) and beta-selinene (-7.1 kcal/mol) had the best binding energies among the screened phytocompounds from the aqueous leaf extract of *O. gratissimum* and performed better than the reference drug. Similar study was done by Noor *et* *al*., (2019) on *in vitro* anti-diabetic, anti-obesity and antioxidant analysis of *Ocimum basilicum* aerial biomass and *in silico* molecular docking simulations with alpha-amylase and lipase enzymes. Alpha-selinene and beta-selinene are sesquiterpenes found in several aromatic plants with encouraging medicinal properties that play a role in diabetes management (de Sousa dos Santos *et al*., 2024). They have been shown to demonstrate excellent antioxidant activity, which helps neutralize reactive oxygen species a significant contributor to pancreatic β-cell destruction and insulin resistance (Nisar *et al*., 2023). Evidence has begun to emerge that alpha- and beta-selinene facilitate peripheral glucose use by modulating key metabolic enzymes, thereby fostering improvement in glycemic control (Arya, 2022). Their lipid-lowering actions might contribute to the protection against cardiovascular complications related to diabetes. Through these combined antioxidant, anti-inflammatory, and metabolic regulatory effects, alpha- and beta-selinene meaningfully contribute to the prevention and attenuation of diabetes progression (de Sousa de Santos *et al*., 2024).

Coupled with lower docked score, the 2D and 3D poses showed that the lead phytocompounds interacted and form bonds such as hydrogen, alkyl and pi-alkyl, carbon-hydrogen, pi-sigma, salt bridge, and van der Waals attraction with key amino acid residues at the binding pockets of the targets. The higher the number of hydrophobic atoms in the active site of the ligand-receptor surface, the higher the binding affinity of ligand-receptor and increased biological activity of the screened compound. Other notable observable bonds such as pi-sigma, pi-Alkyl, and alkyl were known to stabilize the conformation of the ligand intercalating the structure with the binding pocket of the receptor and stabilizing the ligands within the complex (Prabhavathi *et al.*, 2020; Arthur *et al*., 2021).

In addition to the stability of a compound, it is essential to ascertain its effectiveness through its ability to reach the body receptors in appropriate concentration, stays in the bioactive form for as long as it is needed and metabolizes effectively (Nisha *et al.*, 2016; Daina *et al*., 2017). *In silico* ADME is considered in order to preserve time and cost required to find a compound with suitable, acceptable pharmacokinetics and pharmacodynamics properties that are nontoxic at early stage of drug discovery (Nisha et al., 2016; Prasetiawati *et al*., 2019).

During drug discovery process targeted towards oral administration, solubility is one of the main criteria to be considered as it influences absorption. High solubility is also considered for drugs meant for parenteral usage as sufficient number of active constituents need to be delivered in minute quantity (Nisha *et al.*, 2016). According to the SWISSADME prediction server, Log S value indicates solubility, the higher the LogS value, the higher the solubility. Another factor that enhances absorption is the molecular weight (MW), the lower the MW, the higher the rate of the drug absorption. One of the major roles of permeability glycoprotein (P-gp) is to protect the central nervous system. P-gp substrates are responsible for drug efflux from the cell (Nisha *et al.*, 2016).

A CYP450 non-inhibitor is the molecule that will not hinder the biotransformation of drugs metabolized by CYP450 enzyme. During drug breakdown and clearance in the liver, CYP450 super family plays a significant role. CYP450 significant isoforms are CYPIA2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 (Vasanthanatha *et al.*, 2009).

Therefore, the inhibition of cytochrome P450 isoforms might result in drug-drug interactions where co-administered drugs fail to undergo metabolism and mount up to toxic level. It is known that CYP 450 may be vulnerable to modulation by the multiple active constituents of the herbs, because CYPs are subject to induction and inhibition by exposure to varieties of xenobiotics (Zhou *et al.,* 2003).

Lipinski rule-based filter is the most satisfactorily fact of drug-likeness as it is the pioneer rule-of-five. It is defined as the number of hydrogen bond donors <5 (the sum of OHs and NHs), the number of hydrogen bond acceptors <10 (the sum of O, and Ny atoms), the molecular weight < 500 and MLogP< 4.5 (Daina *et al*., 2017). All the phytocompounds exhibit drug-likeness as they all satisfied Lipinski's rule of 5 and do not violate more than one Lipinski's rule.

CONCLUSION

This study demonstrated that the aqueous leaf extract of *Ocimum gratissimum* is a rich source of biological active phytochemicals. The GC-MS analysis confirmed the presence of 27 phytochemicals, while the molecular docking results identified key compounds such as α-selinene, β-selinene with exceptionally high binding affinity for pancreatic lipase. The ability of these phytochemicals found in the aqueous leaf extract of *O. gratissimum* to bind effectively with the active site of the enzyme (pancreatic lipase), may underscore their ability to ameliorate hyperglycaemia. This research therefore provides molecular evidence supporting the antidiabetic and antipacreatic lipase properties of the aqueous leaf extract of *O. gratissimum*.

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APPENDIX

GCMS ANALYSIS PROCEDURE

The extracted sample was analyzed using SHIMADZU GC-2010, equipped with OPTIMA XLB column (60 m X 0.25 mm *i.d.*, 0.25 μm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium was used as the carrier gas at a flow rate of 1 mL/min. Injector and MS transfer line temperatures was set at 220°C and 290°C, respectively. The program was used at 50–150°C at a rate of 3°C/min. Diluted samples was injected in split mode. The components were identified based on the NIST library data of the GC-MS system and literature data (Adams, 2001).

Chromatogram of Aqueous leaf extract of *Ocimum gratissimum* by GC-MS

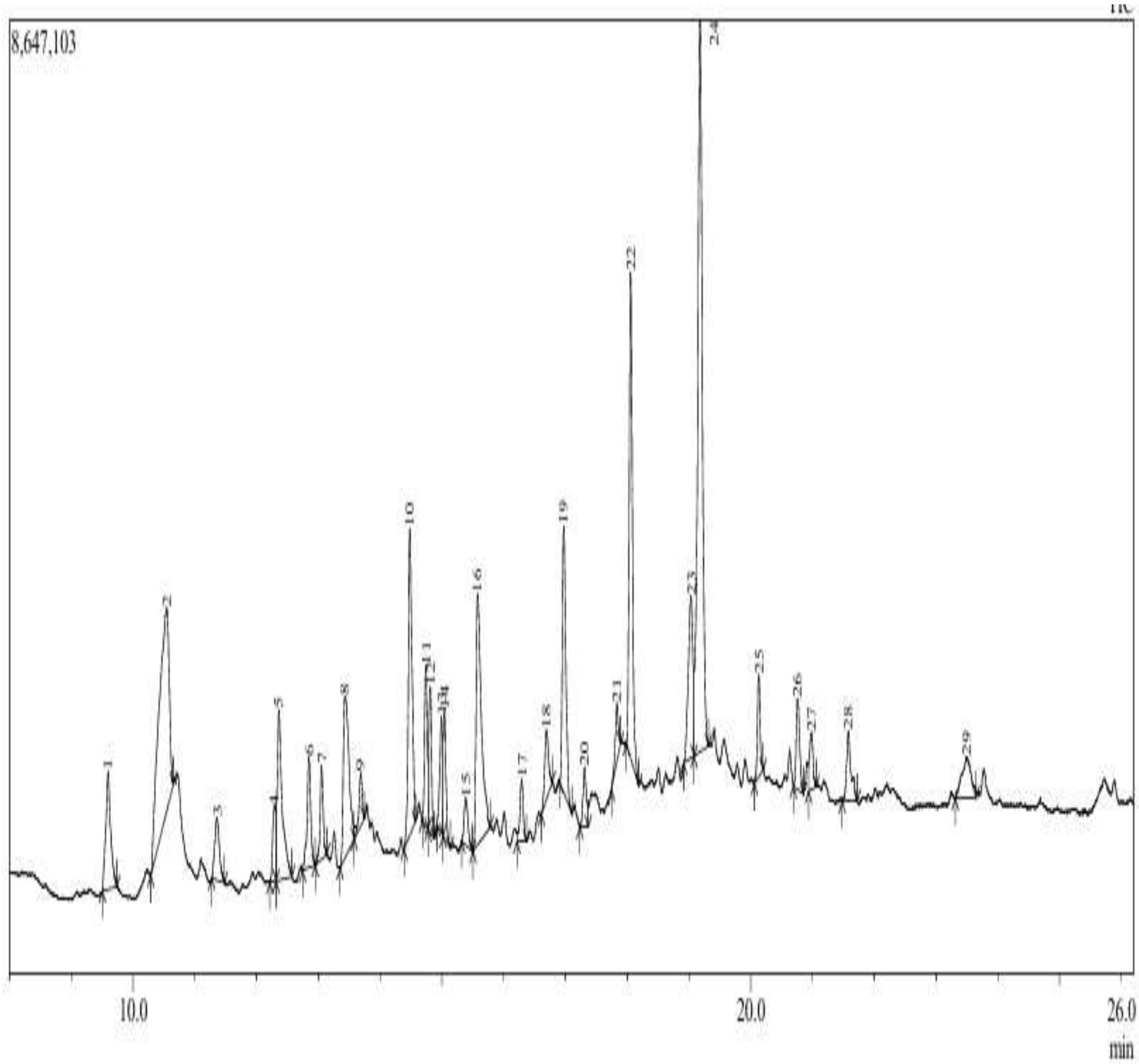

Table 1: Assigned Compounds Detected in the Aqueous leaf extract of *Ocimum gratissimum* from GC-MS Analysis.

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	9.594	5899386	3.05	1070679	2.65	5.51	Cyclohexanone
2	10.542	21989064	11.38	1842985	4.56	11.93	Glycerin
3	11.357	3026216	1.57	565891	1.40	5.35	4-Heptanone, 3-methyl-
4	12.280	2511524	1.30	665447	1.65	3.77	Cyclopropyl carbinol
5	12.361	8612668	4.46	1545797	3.82	5.57	1H-Pyrrole, 2,5-dihydro-
6	12.845	4140194	2.14	1003849	2.48	4.12	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6
7	13.050	2922805	1.51	857392	2.12	3.41	trans-2-Undecen-1-ol
8	13.432	9407003	4.87	1460994	3.61	6.44	Catechol
9	13.685	1874061	0.97	487371	1.21	3.85	Benzofuran, 2,3-dihydro-
10	14.479	11324420	5.86	2783178	6.89	4.07	Thymol
11	14.737	4383417	2.27	1446588	3.58	3.03	1,4-dihydroxy-p-menth-2-ene
12	14.809	3796195	1.97	1322667	3.27	2.87	1,3-Dioxolane, 2,2-dimethyl-4,5-bis(1-methyl
13	14.991	3430607	1.78	1054813	2.61	3.25	2-Methoxy-4-vinylphenol
14	15.040	3271138	1.69	1150903	2.85	2.84	1,3-Dioxolane, 2,2-dimethyl-4,5-bis(1-methyl
15	15.381	1858079	0.96	417640	1.03	4.45	2,7-Octadiene-1,6-diol, 2,6-dimethyl-
16	15.578	13732468	7.11	2272826	5.62	6.04	Acetamide, N-butyl-
17	16.291	2057556	1.07	562656	1.39	3.66	Benzenemethanol, .alpha.,.alpha.,4-trimethyl-
18	16.692	2818267	1.46	628420	1.55	4.48	2-Hydroxy-5-methylisophthalaldehyde

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
19	16.972	9375514	4.85	2419677	5.99	3.87	Benzenemethanol, 4-(methoxymethyl)-.alpha.-
20	17.310	2127584	1.10	537704	1.33	3.96	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]he
21	17.833	1982035	1.03	528907	1.31	3.75	t-Butylhydroquinone
22	18.055	15581668	8.07	4418759	10.93	3.53	1,4-Dimethoxy-2,3-dimethylbenzene
23	19.031	7666153	3.97	1467530	3.63	5.22	Bicyclo[2.2.1]heptane-2,3-diol, 1,7,7-trimethy
24	19.179	34213605	17.71	6637872	16.42	5.15	Supraene
25	20.129	2753335	1.43	925526	2.29	2.97	Phytol, acetate
26	20.761	3206233	1.66	822815	2.04	3.90	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethy
27	20.981	2027072	1.05	515704	1.28	3.93	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-me
28	21.581	3234104	1.67	634608	1.57	5.10	2,6,6-Trimethyl-3-(phenylthio)cyclohept-4-enc
29	23.489	3919269	2.03	368204	0.91	10.64	2-methyloctacosane
		193141640	100.00	40417402	100.00		

Table 2: SMILES of the aqueous leaf extract of *Ocimum gratissimum*

COMPOUND NAME	PUBCHEM CID	SMILES
Catechol	289	C1=CC=C(C(=C1)O)O
2-Methoxy-4-vinylphenol	332	COC1=C(C=CC(=C1)C=C)O
Glycerin	753	C(C(CO)O)O
Thymol	6989	CC1=CC(=C(C=C1)C(C)C)O
Cyclohexanone	7967	C1CCC(=O)CC1
Benzofuran, 2,3-dihydro-	10329	C1COC2=CC=CC=C21
t-Butylhydroquinone	16043	CC(C)(C)C1=C(C=CC(=C1)O)O
4-Heptanone, 3-methyl-	27470	CCCC(=O)C(C)CC
Acetamide, N-butyl-	61265	CCCCNC(=O)C
Cyclopropyl carbinol	75644	C1CC1CO
2-Hydroxy-5-methylisophthalaldehyde	81744	CC1=CC(=C(C(=C1)C=O)O)C=O
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	119838	CC1=C(C(=O)C(CO1)O)O
1,4-Dimethoxy-2,3-dimethylbenzene	148299	CC1=C(C=CC(=C1C)OC)OC
7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-	232703	CC12CCC(CC1O2)C3(CO3)C
1,4-dihydroxy-p-menth-2-ene	300085	CC(C)C1(CCC(C=C1)(C)O)O
1,3-Dioxolane, 2,2-dimethyl-4,5-bis(1-methylethenyl)-	539045	CC(=C)C1C(OC(O1)(C)C)C(=C)C
2,6,6-Trimethyl-3-(phenylthio)cyclohept-4-enol	585064	CC1C(CC(C=CC1SC2=CC=CC=C2)(C)C)O
Benzenemethanol, 4-(methoxymethyl)-alpha.-methyl-	591895	CC(C1=CC=C(C=C1)COC)O

Benzenemethanol, alpha.,alpha.,4-trimethyl-	601306	CC1=CC(=C(C=C1OC)C(C)(C)O)O
Supraene	638072	CC(=CCC/C(=C/CC/C(=C/CC/C=C(/CC/C=C(/CCC=C(C)C)\C)\C)/C)/C)C
2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	5280662	CC1=CC(=O)CC(C1(/C=C/C(=O)C)O)(C)C
2,7-Octadiene-1,6-diol, 2,6-dimethyl-	5280678	C/C(=C\CCC(C)(C=C)O)/CO
trans-2-Undecen-1-ol	5365004	CCCCCCCC/C=C/CO
Bicyclo[2.2.1]heptane-2,3-diol, 1,7,7-trimethyl-	169447117	[2H]C([2H])([2H])[C@]1([C@H]2CC[C@@]([C@@H]1O)(C2(C)C)C)O
2-methyloctacosane	519147	CCCCCCCCCCCCCCCCCCCCCCCCCC(C)C
Alpha-selinene	10856614	CC1=CCC[C@]2([C@H]1C[C@@H](CC2)C(=C)C)C
Beta-selinene	442393	CC(=C)[C@@H]1CC[C@]2(CCCC(=C)[C@@H]2C1)C