Arab American University
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Abelmoschus Esculentus, Hypericum Triquetrifolium, Ocimum Basilicum, and Gundelia Tournefortii Antidiabetic Plants Extract Regulates the Expression of Genes Involved in Glucose Uptake and Insulin Signaling Cascade in L6 myc GLUT4 Rat's Cells.

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Declaration

I declare that, except where explicit reference is made to the contribution of others, this thesis is substantially my own work and has not been submitted for any other degree at the Arab American University or any other institution.

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Dedication

This thesis is dedicated to my parents, who have been my biggest source of strength through their love, sacrifices, and unending support. I've been led every step of the way by your assistance. I am grateful to my sisters (Liali, Farah, Tamara, Zeina, Shaima) and my brother Suliman for their love, support, and faith in me. And a particular thanks to my sister Nour, whose wisdom and support have sustained me through difficult times. I appreciate my colleagues' collaboration and encouragement. The experience of working with you has been wonderful. I also want to express my gratitude to my best friend Rawand, thank you for always being there, cheering me on, and believing in me. This work is dedicated to all of you, your support has meant the world to me.

Sincerely,
Sally Afif Suliman Al-Joudeh

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Abstract

Hyperglycemia and insulin resistance are hallmarks of type 2 diabetes mellitus, a chronic illness mainly brought on by abnormalities in skeletal muscle insulin signaling. Hyperglycemia is made worse by malfunctions in important proteins that control glucose transport, including IRS1, AS160 ,PTEN and GLUT4. For treatments to be effective beyond symptom control, these molecular abnormalities must be addressed. Using RT-PCR, the potential of methanolic extracts of four medicinal herbs Ocimum basilicum(OB), Abelmoschus esculentus (AE), Gundelia tournefortii(GT) and Hypericum triquetrifolium(HT) to enhance insulin signaling in L6 myoblast cells is being investigated. Since the extracts maintained cellular integrity at the recommended dosages (0–1000µg/ml), cell viability studies demonstrated that they were safe for therapeutic use. According to experimental results of the mRNA expression level for each of the important proteins described, HT significantly increased GLUT4 expression, suggesting greater glucose absorption, while GT lowered GLUT4 expression, suggesting probable blockage. GT, OB, and AE down regulated PTEN expression in the presence of insulin, potentially enhancing insulin signaling. Further evidence that AE contributes to the reversal of insulin resistance comes from the restoration of IRS1 and AS160 expression. To further investigate the molecular mechanisms underlying AE’s effects, HPLC analysis was conducted confirmed the presence of bioactive compounds with hypoglycemic, antioxidant, and anti-inflammatory properties, such as ferulic acid, quercetin, caffeic acid, benzoic acid, vanillic acid, and chlorogenic acid. Following their isolation using preparative HPLC, it was discovered that caffeine and chlorogenic acid played a significant role in the insulin-sensitizing effects of these phenolic compounds. The mRNA expression analysis revealed that phenolic compounds from AE significantly downregulated PTEN while upregulating GLUT4, IRS1 and AS160 expression. These findings indicate a potential link between AE's insulin-sensitizing properties and its phenolic composition. This study finds that medicinal plants are cost-effective ways to manage type 2 diabetes by molecularly addressing insulin resistance. The findings support their traditional use and promote the creation of plant-based antidiabetic medications that address the fundamental causes of the disease.
Keywords: Type 2 diabetes, Insulin signaling, Glucose transport, Medicinal plants, Phytochemicals

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List of Definitions of Abbreviations

Abbreviations	Title
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
MODY	Maturity-Onset Diabetes of the Young
IDF	International Diabetes Federation
NCDs	Non-Communicable Diseases
HbA1c	Hemoglobin A1c
NAFLD	Nonalcoholic Fatty Liver Disease
GLUT	Glucose Transporter
AMPK	AMP-Activated Protein Kinase
GLUT4	Glucose Transporter Type 4
T2D	Type 2 Diabetes
IRS	Insulin Receptor Substrate
IRS1	Insulin Receptor Substrate 1
PI3K	Phosphoinositide 3-Kinase
Akt	Protein Kinase B
PTEN	Phosphatase and Tensin Homolog
PDK1	3-Phosphoinositide-Dependent Protein Kinase 1
PIP2	Phosphatidylinositol 4,5-Bisphosphate
PIP3	Phosphatidylinositol (3,4,5)-Triphosphate
ERK	Extracellular Signal-Regulated Kinase

List of Definitions of Abbreviations

AS160	Akt Substrate of 160 kDa
aPKC	Atypical Protein Kinase C
PTP1B	Protein Tyrosine Phosphatase 1B
ROS	Reactive Oxygen Species
NF-κB	Nuclear factor kappa B
NrF2	Nuclear erythroid 2-related factor 2
A.E	Abelmoschus Esculentus
G.T	Gundelia Tournefortii
H.T	Hypericum Triquetrifolium
O.B	Ocimum Basilicum
GLP-1	Glucagon-like peptide-1
DPP-4	Dipeptidyl peptidase-4
SGLT-2	Sodium-glucose cotransporter-2
CAM	Complementary and Alternative Medicine
WHO	World Health Organization
CA	Caffiec Acid
CA	Chlorogenic acid
FA	Ferulic Acid
VA	Vanillic Acid
BA	Benzoic Acid

Chapter One: Introduction

The primary aim of this study was to investigate the effect of selected antidiabetic plant extracts on the expression of key proteins involved in the insulin signaling pathway, including insulin receptor substrate-1 (IRS1), Akt Substrate of 160 kDa (AS160), Phosphatase and Tensin Homolog (PTEN), and Glucose Transporter Type 4 (GLUT4). This was achieved by examining the mRNA expression levels of these proteins in L6 GLUT4 cells treated with plant extracts from Abelmoschus esculentus, Hypericum triquetrifolium, Ocimum basilicum, and Gundelia tournefortii. These plants, previously evaluated for their antidiabetic efficacy (Mann, Riddell, and Adegoke 2022; Haque et al. 2022; Kadan et al. 2021a), are known to enhance glucose uptake in L6 GLUT4 cells. The study also focuses on the active compounds within these extracts, such as chlorogenic acid, ferulic acid, quercetin, caffeic acid, vanillic acid, and benzoic acid, due to their potential therapeutic properties. To achieve this, RT-PCR was employed to assess and analyze gene expression levels. Given the novelty of these medicinal plants and the limited existing research, this study seeks to address numerous research gaps, with each new finding contributing valuable insights to the field of antidiabetic natural compounds.

Importance of the study:

Type 2 diabetes mellitus (T2D), a progressive and multifaceted disorder, poses one of the most significant global health challenges of the 21st century. Effective long-term management remains difficult, with the disease often leading to severe complications such as cardiovascular disease, diabetic nephropathy, and retinopathy due to sustained high blood glucose levels. A major factor contributing to the development of T2D is insulin resistance in skeletal muscle. This study underscores the critical need to understand the molecular mechanisms underlying T2D. Disruptions in the insulin signaling pathway, involving key proteins such as IRS1, AKT, AS160, and GLUT4, play a pivotal role in the onset of insulin resistance. Investigating these disruptions offers valuable insights into the disease's biology, enabling the identification of therapeutic targets and the development of treatments that restore normal signaling and enhance glucose uptake in skeletal muscle.

Furthermore, the study highlights the potential of natural compounds derived from medicinal plants as therapeutic agents. These bioactive compounds interact with specific molecular pathways, providing opportunities to validate and optimize their use in diabetes management. By identifying the most effective compounds like (ferulic acid, caffiec acid, chlorogenic acid, benzoic acid vanillic acid and quercetin) which Studies have linked them to improved insulin sensitivity, by reducing oxidative stress, inflammation, and glucose spikes while improving glucose uptake and metabolism also understanding their mechanisms of action, this research can guide the development of safer and more efficient plant-based remedies. Molecular-level investigations also facilitate the creation of groundbreaking therapies, such as gene editing, RNA-based treatments, and small-molecule inhibitors, capable of addressing the root causes of diabetes rather than merely managing its symptoms. By bridging the gap between foundational science and clinical applications, this research holds the potential to revolutionize diabetes care, offering precise, innovative, and effective solutions to improve patient outcomes worldwide.

Research Problem:

Type 2 diabetes mellitus (T2D) is a complex and progressive disorder that presents significant challenges in effective long-term management. Despite advancements in treatment, the disease remains a leading global health issue, often resulting in severe complications such as cardiovascular disease, diabetic nephropathy, and retinopathy due to chronic hyperglycemia. Insulin resistance in skeletal muscle, a primary site for glucose uptake, is a key contributor to the pathophysiology of T2D. Current therapeutic approaches primarily focus on symptom management rather than addressing the root causes of the disease. A deeper understanding of the molecular mechanisms involved in insulin resistance particularly disruptions in the insulin signaling pathway mediated by key proteins such as IRS1, AKT, AS160, and GLUT4 is crucial for identifying novel therapeutic targets. Additionally, while natural compounds derived from medicinal plants show promising antidiabetic potential, their mechanisms action remain insufficiently explored. There is a critical need to validate and optimize the use of these bioactive compounds to develop safer and more effective plant-based therapies. This research aims to address these gaps by investigating the molecular mechanisms underlying T2D and evaluating the therapeutic potential of bioactive compounds in medicinal plants. By bridging the gap between molecular science and clinical application, the study seeks to contribute to the development of innovative, precise, and sustainable therapies to improve outcomes for individuals with T2D.

Aim of the study:

1. To identify how Abelmoschus esculentus, Hypericum triquetrifolium, Ocimum basilicum, and Gundelia tournefortii affect the expression of key proteins in the insulin signaling pathway, particularly their role in GLUT4 mobilization.
2. To isolate and evaluate the efficacy of bioactive compounds in Abelmoschus esculentus, efficacy will be assessed through mRNA gene expression analysis following RNA isolation in L6 myc GLUT4 cells.

Research Questions:

1. What are the optimal concentrations of each plant extract that minimize cytotoxicity while maintaining cellular viability?
2. What are the mRNA expression levels of key proteins in the insulin signaling pathway (e.g., IRS1, AS160, PTEN, and GLUT4) responsible for enhancing GLUT4 translocation to the skeletal muscle plasma membrane following treatment with antidiabetic plant extracts (Abelmoschus esculentus, Hypericum triquetrifolium, Ocimum basilicum, and Gundelia tournefortii*)*?
3. Which active compounds within Abelmoschus esculentus extracts exhibit the highest antidiabetic activity, contributing most effectively to glucose uptake in skeletal muscle cells and their mRNA expression levels?

Research hypothesis:

1. Primary Hypothesis is that treatment with plant extracts from Abelmoschus esculentus, Hypericum triquetrifolium, Ocimum basilicum, and Gundelia tournefortii will change the expression of key proteins involved in the insulin signaling pathway, including IRS1, AS160, PTEN, and GLUT4, in L6 GLUT4 cells causing an increase in GLUT4 translocation to skeletal muscle plasma membrane.
2. Secondary Hypotheses that the active compounds present in the plant extracts contribute significantly to the upregulation of these proteins.

Research Limitations:

Research problems in laboratory techniques span a range of challenges that can affect experimental outcomes. In cell viability assays, such as the MTT assay, ensuring uniform seeding of cells in microtiter plates and mitigating edge effects is a critical concern. During RNA extraction and cDNA synthesis, contamination of RNA with proteins or solvents poses a significant challenge, potentially compromising the quality and accuracy of downstream applications. Similarly, in analytical HPLC and preparative HPLC analysis, retention time drift or poor resolution during chromatographic analysis can hinder the reproducibility and reliability of results. Addressing these issues is essential for improving experimental consistency and data quality.

Keyword:

Diabetes Mellitus (DM): A metabolic disorder characterized by abnormally high blood glucose levels, with subtypes such as type 1 (T1DM) and type 2 (T2DM) diabetes.(Rajaei et al. 2019)

Type 1 Diabetes Mellitus (T1DM): A form of diabetes where the body's immune system destroys pancreatic beta cells, leading to insufficient insulin production. It is commonly diagnosed in children or adolescents.(Ruze et al. 2023)

Type 2 Diabetes Mellitus (T2DM): A form of diabetes primarily linked to insulin resistance, often caused by poor diet and inactivity, affecting primarily middle-aged and older adults.(Kazerouni et al. 2020)

Hyperglycemia: Elevated blood glucose levels, commonly associated with diabetes.(Galicia-Garcia et al. 2020)

Insulin Resistance: A condition where the body's tissues, especially skeletal muscle, become less responsive to insulin, leading to impaired glucose uptake, elevated blood glucose levels, and increased risk of Type 2 diabetes.(Merz and Thurmond 2020)

Insulin: A peptide hormone that helps regulate blood glucose by facilitating glucose uptake by cells.(Harris and Johnson 2019)

Glucose Homeostasis: The body's mechanism for maintaining stable blood glucose levels through processes such as insulin secretion, glucose uptake, and glucose production.(Zhang et al. 2019)

Glucose Transporters (GLUTs): Membrane proteins that facilitate glucose entry into cells. GLUT4, in particular, plays a key role in insulin-regulated glucose uptake in tissues like muscle and adipose tissue.(Ojo et al. 2023)

GLUT4 (Glucose Transporter Type 4): A major glucose transporter in skeletal muscle, responsible for insulin-regulated glucose uptake, crucial for maintaining glucose homeostasis and energy balance.(McMillin et al. 2017)

Glycogenesis: The process of glucose storage in the form of glycogen in the liver and muscles.(Milionis et al. 2023)

Glycogenolysis: The breakdown of stored glycogen into glucose, typically initiated during fasting or low blood glucose levels.(Dall'Agnese et al. 2022)

GLUT4 Translocation: The process by which GLUT4 glucose transporters move to the cell surface in response to insulin, allowing glucose uptake by cells.(Ojo et al. 2023)

Metabolic Disorders: Disorders that disrupt normal metabolic processes, often related to abnormal glucose regulation, like T2DM.(Chawla, Chawla, and Jaggi 2016)

Skeletal Muscle: A highly insulin-sensitive tissue where most insulin-stimulated glucose utilization occurs, playing a key role in glucose uptake, storage as glycogen, and energy production.(Giacometti et al. 2020)

Insulin Receptor: A glycoprotein on the cell membrane that binds insulin and triggers signaling pathways involved in glucose uptake, glycogen synthesis, and protein metabolism.(Mann, Riddell, and Adegoke 2022)

Insulin Receptor Substrates (IRS): Proteins involved in insulin signaling, crucial for the transduction of insulin's effects on glucose metabolism. IRS-1 is particularly important in skeletal muscle for glucose uptake.(Mann, Riddell, and Adegoke 2022)

PI3K/Akt Pathway: A critical signaling cascade activated by insulin, leading to GLUT4 translocation to the plasma membrane, enhancing glucose uptake in skeletal muscle. Disruptions in this pathway are linked to insulin resistance and Type 2 diabetes.(Shanak et al. 2022; Rajendiran et al. 2024)

AS160 (Akt Substrate of 160 kDa): A key protein involved in GLUT4 translocation. Its phosphorylation is necessary for glucose uptake in skeletal muscle.(Shanak et al. 2022)

Phosphatases (PTP1B and PTEN): Enzymes that negatively regulate insulin signaling by dephosphorylating key molecules, exacerbating insulin resistance in Type 2 diabetes.(Al-Ishaq et al. 2019)

Herbal Medicine: The use of plants or plant extracts for therapeutic purposes. It is a key component of complementary and alternative medicine (CAM) and is gaining popularity due to its perceived safety and effectiveness.(Ahda et al. 2023)

Anti-diabetic Properties: The ability of certain plants or compounds to reduce blood glucose levels and manage or prevent diabetes, typically through mechanisms such as enhancing insulin secretion or reducing glucose absorption.(Owusu et al. 2020)

Phytochemicals: Bioactive compounds found in plants, which have therapeutic properties. These include secondary metabolites like flavonoids, alkaloids, terpenes, and phenolics, which contribute to the medicinal effects of plants.(Huo and Qian 2018)

Medicinal Plants: Plants that contain bioactive compounds capable of treating or preventing diseases. These plants are widely studied for their potential to manage chronic conditions like diabetes.(Zaid et al. 2017)

Bioactive Compounds: Natural chemical substances found in plants that have biological effects on the human body. Examples include alkaloids, flavonoids, tannins, and phenolics.(Zaid et al. 2017)

Flavonoids: A group of plant secondary metabolites known for their antioxidant, anti-inflammatory, and anti-diabetic effects.(Sereno et al. 2022)

Non-flavonoid Phenolics: A subgroup of phenolic compounds that do not belong to the flavonoid category. These include phenolic acids, quinones, stilbenes, and coumarins, which contribute to anti-diabetic and other health benefits.(Akhlaghipour et al. 2023)

Phenolic Compounds: A class of bioactive molecules found in plants, recognized for their antioxidant and anti-inflammatory properties. They are considered crucial for protecting plants and offer various health benefits when consumed.(Kadan et al. 2021b)

Medicinal Plant Extracts: Concentrated forms of medicinal plants that contain active ingredients used for therapeutic purposes.(Sitobo, Navhaya, and Makhoba 2024)

Chlorogenic Acid (CA): A phenolic compound found in coffee and several plant-based foods, known for its ability to improve insulin sensitivity, regulate glucose metabolism, and offer antioxidant and anti-inflammatory effects.(Yan et al. 2020)

Caffeic Acid (CA): A non-flavonoid phenolic compound with antioxidant properties that can improve insulin secretion and reduce insulin resistance, helping manage diabetes. (Akhlaghipour et al. 2023)

Ferulic Acid (FA): A phenolic compound with antioxidant and antihyperglycemic properties. It helps control blood sugar levels and prevent complications of diabetes, such as lipid peroxidation and pancreatic tissue damage.(Salau et al. 2023)

Quercetin: A flavonoid with anti-diabetic effects, improving insulin production, and influencing several signaling pathways associated with diabetes pathogenesis. It is also known for its ability to regenerate pancreatic islets and reduce insulin resistance.(Chellian et al. 2022)

Vanillic Acid (VA): A phenolic compound with antioxidant and antimicrobial properties, also noted for its antitumor, anticlastogenic, and antimutagenic effects. It is considered a potential nutraceutical for managing diabetes.(Singh et al. 2022)

Benzoic Acid: Benzoic acid is a colorless, crystalline aromatic carboxylic acid (C7H6O2) commonly used as a preservative in food and cosmetics due to its antimicrobial properties. It is naturally found in various plants and also serves as a precursor in the synthesis of numerous chemicals.(Sun and Shahrajabian 2023)

Chapter Two: Literature Review

2.1 Diabetes Mellitus (DM)

Diabetes mellitus derives its name from the Greek word *diabetes,* meaning "to siphon" or "pass through," and the Latin word *mellitus,* meaning "sweet." It is a metabolic disorder characterized by abnormally high blood glucose levels. DM is classified into several categories, including type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), maturity-onset diabetes of the young (MODY), and gestational diabetes. Among these, T1DM and T2DM are the most common subtypes. T1DM typically results from insufficient insulin production due to autoimmune destruction of pancreatic beta cells and often manifests in children or adolescents. In contrast, T2DM, commonly associated with insulin resistance, is primarily linked to prolonged hyperglycemia caused by poor dietary habits and an inactive lifestyle. It usually affects middle-aged and older adults.(Rajaei et al. 2019) The underlying causes, symptoms, and treatment approaches for T1DM and T2DM differ significantly.

According to the International Diabetes Federation (IDF), nearly half of all people with diabetes (240 million), predominantly T2DM, remain undiagnosed. In 2021, the global prevalence of diagnosed diabetes was estimated at 537 million, with projections rising to 643 million by 2030 and 783 million by 2045 (Ruze et al. 2023). T2DM is among the most widespread chronic non-communicable diseases (NCDs) worldwide (Kazerouni et al. 2020), It is the third leading cause of death due to disease and complications, the second leading cause of mortality overall, and significantly increases the risk of cardiovascular disease (fivefold) and stroke (threefold)(Okoronkwo et al. 2015).

2.1.1 Symptoms and Complications of Type 2 Diabetes

Type 2 diabetes is characterized by symptoms such as frequent urination (*polyuria*), increased hunger (*polyphagia*), excessive thirst (*polydipsia*), unexplained weight loss, and, in severe cases, unconsciousness. If left unmanaged, diabetes can result in serious complications, including nephropathy, atherosclerosis, and damage to major organs like the heart, nerves, kidneys, and eyes(Chawla, Chawla, and Jaggi 2016). T2DM is one of the most prevalent metabolic disorders, primarily caused by two factors: defective insulin secretion from pancreatic β-cells and the reduced responsiveness of insulin-sensitive tissues, resulting in elevated blood glucose levels (*hyperglycemia*) (Chawla, Chawla, and Jaggi 2016). Insulin production and action must precisely match the body's metabolic demands. This requires tight regulation of molecular mechanisms involved in insulin synthesis, release, and tissue responsiveness. Any disruption in these mechanisms can lead to metabolic imbalances, ultimately contributing to the development of T2DM. (Galicia-Garcia et al. 2020)

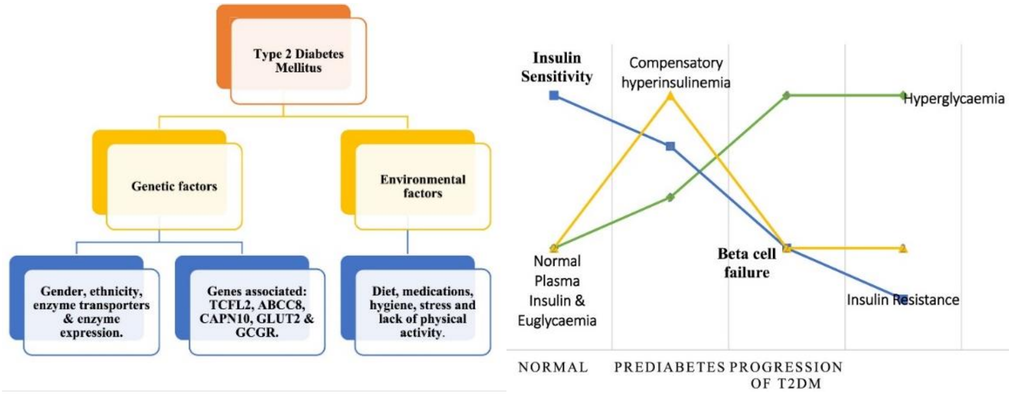

Figure 2.1: Factors influencing type 2 diabetes mellitus.(Ojo et al. 2023)

2.1.2 Glucose Homeostasis and Metabolic Regulation

Glucose is the primary energy source for human cells, derived from dietary intake, processed in the body, and transported from the bloodstream into cells. The transport of glucose across cell membranes is a critical biological process (Leszek 2019). The breakdown of macronutrients carbohydrates, lipids, and proteins eventually yields glucose, which powers the body's metabolic activities. The key processes involved in glucose metabolism include glycogenesis, glycolysis, gluconeogenesis, and glycogenolysis. Glycolysis, an enzyme-mediated metabolic pathway, facilitates the breakdown of glucose within cells, producing pyruvate as an end product (Harris and Johnson 2019). To maintain stable blood glucose levels during fasting, hepatocytes perform a series of reactions in the cytosol and mitochondria, synthesizing glucose from non-carbohydrate sources. This process is regulated by hormones such as insulin, glucagon, and cortisol (Zhang et al. 2019).

During fasting, glucagon released by the pancreas initiates glycogenolysis, the breakdown of stored glycogen into glucose and glucose-1-phosphate to meet the body's energy demands. On the other hand, glycogenesis is the process of glycogen synthesis, where glucose molecules are linked together for storage in the liver and muscles. These mechanisms collectively ensure a steady supply of glucose for cellular energy. In humans, glucose metabolism follows a circadian rhythm, with glucose tolerance being highest in the morning, this is largely because pancreatic beta cells are most active during the early hours of the day, while glycogen storage peaks in the evening. After midday, adipose tissue becomes more sensitive to insulin, reflecting the body's varying energy utilization patterns throughout the day and these fluctuations form the basis of the glucose metabolism cycle.

Under normal conditions, fasting blood sugar levels after 3–4 hours without eating typically range from 80 to 90 mg/dL. Following a meal, blood sugar may rise to 120–140 mg/dL, but the body's feedback mechanisms usually restore glucose levels to normal within two hours. During prolonged fasting or starvation, the process of gluconeogenesis ensures a continuous supply of glucose. Elevated blood glucose levels trigger the secretion of insulin, facilitating the movement of glucose from the extracellular space into cells, thereby reducing blood glucose. Conversely, low blood glucose stimulates glucagon release, which works to elevate glucose levels by mobilizing stored energy reserves. A disruption in this finely tuned balance, such as insufficient or impaired insulin secretion, results in conditions like diabetes mellitus (Ojo et al. 2023).

The term "glucose homeostasis" (also known as glycemic control or glucose metabolism) describes the body's overall mechanism for regulating blood sugar levels. The key factor affecting this balance is insulin sensitivity, particularly in muscle tissue. Impaired insulin sensitivity is a significant defect that can lead to disruptions in glucose homeostasis (Milionis et al. 2023).

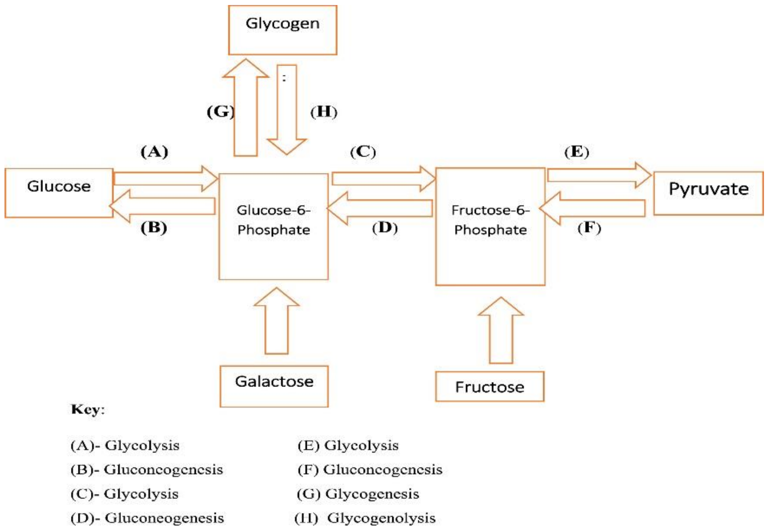

Figure 2.2: Diagram representing the relationship among the processes of glucose metabolism.(Ojo et al. 2023)

2.1.3 Insulin Resistance: Mechanisms and Implications

Insulin is a peptide hormone composed of 51 amino acids, consisting of an α and a β chain connected by two interchain disulfide bonds and a third intrachain disulfide bond within the α chain. Both external and internal cellular factors can impair the cellular response to insulin, leading to insulin resistance a condition commonly associated with type 2 diabetes (T2D) (Dall'Agnese et al. 2022). Insulin resistance is characterized by reduced sensitivity to insulin, often accompanied by increased insulin secretion to compensate (Li et al. 2022), this condition is marked by a diminished ability of target tissues to respond to insulin, which impairs glucose disposal, inhibits glycogen synthesis, promotes lipolysis, and disrupts the regulation of hepatic glucose production (Petersen & Shulman, 2018). Initially, increased insulin secretion results in hyperinsulinemia, which is traditionally viewed as a compensatory response. However, if left unchecked, insulin resistance can contribute to β-cell dysfunction over time.

Lifestyle interventions such as weight loss, regular exercise, and balanced nutrition can often reverse these defects, and without such interventions, insulin resistance may progress, leading to metabolic disorders such as type 2 diabetes, characterized by fasting and postprandial hyperglycemia, elevated HbA1c levels, and nonalcoholic fatty liver disease (NAFLD), often accompanied by hyperinsulinemia. However, recent research using genetically modified mouse models has questioned the direct causal relationship between insulin resistance and T2D (da Silva Rosa et al. 2020). The mechanisms underlying insulin resistance in diabetes include abnormalities in insulin production, impaired post-receptor signaling (the most common cause), mutations in insulin receptors, and the presence of insulin antagonists. A key metabolic defect in T2D is the down regulation of GLUT4 translocation, which reduces glucose uptake by target cells (Al-Ishaq et al. 2019).

2.1.4 Glucose transport and defect in transport activity

Glucose transport is a tightly regulated process that occurs via facilitated diffusion, utilizing specialized carrier proteins to cross cell membranes. Among the various glucose transporters, the most significant are GLUT1 to GLUT5, which are responsive to metabolic cues such as growth factors, hypoglycemia, stress hormones, and hypoxia. These transporters are regulated through multiple signaling pathways and play a critical role in delivering glucose to cells for energy production and metabolic processes. In certain conditions, glucose transport proteins may undergo alterations in structure, expression, localization, synthesis, or function due to factors such as hormonal imbalances, toxins, kinase signaling pathways, or inflammation. These changes can impair glucose transport and exacerbate metabolic disorders like diabetes (McCall 2019). Defective or insufficient glucose transport is a key contributor to impaired glucose tolerance and the pathogenesis of type 2 diabetes.

A crucial focus in understanding reduced glucose transport and disposal in type 2 diabetes lies in the role of GLUT4, an insulin-dependent transporter. In individuals with type 2 diabetes, defective glucose uptake is observed in the brain, visceral adipose tissue, and skeletal muscles. These impairments are among the most accurate markers of insulin resistance (Olson and Humphries, 2020). Addressing these transport defects is essential for managing and mitigating the progression of insulin resistance and related metabolic diseases.(Olson and Humphries 2020).

2.1.5 The Role of GLUT4 in Glucose Transport and Metabolic Regulation

GLUT4, a glucose transporter, is predominantly expressed in insulin-sensitive tissues, including skeletal muscle, liver, and adipose tissue. It dynamically cycles between the plasma membrane and intracellular compartments. Under basal conditions, GLUT4 remains sequestered within intracellular vesicles, rendering it inaccessible to extracellular glucose. However, upon insulin stimulation or muscle contraction, GLUT4 translocate to the cell surface, facilitating the uptake of glucose from the extracellular environment into the cell. GLUT4 plays a critical role in insulin-regulated glucose uptake, making it a key component of normal glucose homeostasis. Its function is particularly significant in type 2 diabetes mellitus (T2DM) and insulin resistance, where impaired GLUT4 activity disrupts blood glucose regulation.

GLUT4 is the primary transporter responsible for reducing blood glucose levels following its translocation, underscoring its importance in managing metabolic health. As a result, GLUT4 has been extensively studied in diabetic models. Both insulin signaling and physical activity trigger pathways that drive GLUT4 translocation to the plasma membrane. Insulin activates its receptor and downstream signaling cascades, while physical activity stimulates the AMP-activated protein kinase (AMPK) pathway, both converging to enhance GLUT4 activity. This dual regulation highlights the potential of targeting GLUT4 in therapeutic strategies for T2DM and related metabolic disorders (Shanak et al. 2022).

2.1.6 GLUT4: A Key Regulator of Glucose Uptake in Skeletal Muscle

GLUT4 is the predominant glucose transporter in skeletal muscle and plays a critical role in regulating glucose uptake and metabolism. Numerous studies have established a strong positive correlation between GLUT4 protein levels and the efficiency of muscle glucose uptake. Research using mouse skeletal muscle models has shown that overexpression of GLUT4 significantly enhances glucose uptake under various conditions, increasing basal uptake by 20–300%, insulin-stimulated uptake by 60–200%, and contraction-induced uptake by 35%.

Conversely, the targeted loss of GLUT4 in skeletal muscle results in a 70–80% reduction in basal glucose uptake and a complete inhibition of glucose uptake induced by insulin or muscle contraction. These findings highlight the indispensable role of GLUT4 in facilitating glucose uptake in skeletal muscle during short-term stimuli (McMillin et al. 2017). Additionally, GLUT4 is the most abundant glucose transporter in skeletal muscle and is regarded as a rate-limiting factor for glucose uptake and metabolism, particularly in the resting state of the muscle. This underscores its pivotal role in maintaining glucose homeostasis and its potential as a therapeutic target for metabolic disorders (Chadt and Al-Hasani 2020).

2.1.7 Skeletal Muscle

Skeletal muscle is a highly insulin-sensitive tissue responsible for glucose disposal through the regulated activity of glucose transporter 4 (GLUT4). In diabetes, impaired insulin signaling and disruptions in GLUT4 vesicle trafficking contribute to defects in insulin-stimulated GLUT4 translocation, hindering efficient glucose uptake (Giacometti et al. 2020). Approximately 90% of insulin-stimulated glucose utilization occurs in skeletal muscle, underscoring its crucial role in glucose metabolism and overall energy balance (Huang et al. 2018). During both exercise and insulin stimulation, skeletal muscle serves as the primary site for glucose uptake, utilization, and storage in the form of glycogen.

Glycogen, the stored form of carbohydrates in humans and other mammals, consists of up to 50,000 glucose units linked by α (1→4) bonds and branched via α (1→6) bonds. In humans, approximately 80% of glycogen is stored in skeletal muscle, which accounts for 40–50% of body weight in healthy young adults. Glycogen concentrations in muscle typically range from 80 to 150 mmol per kilogram of wet weight. While a key function of glycogen is to support energy production during exercise, skeletal muscles lack glucose-6-phosphatase and are therefore unable to release glucose into the bloodstream. As a result, muscle glycogen primarily levels during fasting. However, muscle glycogen can be metabolized into lactate, which can be transported to the liver. Through gluconeogenesis, the liver can then convert lactate into glucose, aiding in the maintenance of normal blood glucose levels (euglycemia)(Jensen et al. 2011).

From an evolutionary perspective, the primary function of skeletal muscle glycogen is believed to be its role as an immediate energy reserve during "fight or flight" situations. This rapid energy availability would have been crucial for survival in response to acute threats or emergencies. Notably, reduced glycogen levels in skeletal muscles are associated with increased insulin sensitivity, a phenomenon likely linked to the body's drive to restore glycogen stores quickly in preparation for future challenges. Intracellular glycogen serves as a readily accessible energy source, with a production rate that significantly surpasses the flux of glucose uptake into skeletal muscles. This rapid energy production capability positions muscle glycogen as a critical substrate for high-intensity and immediate energy demands. While glycogen provides a localized energy source for acute responses, accumulated fat serves as a longer-term energy reserve, supporting survival during periods of starvation. This dual energy-storage strategy underscores the evolutionary balance between short-term survival mechanisms and sustained energy needs, highlighting the complementary roles of glycogen and fat in human physiology(Jensen et al. 2011).

2.1.7 Insulin Resistance in Skeletal Muscle: An Early Marker of Type 2 Diabetes

Insulin resistance in skeletal muscle arises from reduced sensitivity to insulin secreted by the pancreas, impairing glucose uptake and leading to elevated blood glucose levels. This condition can precede β-cell dysfunction and the clinical onset of type 2 diabetes (T2D) by several decades. Evidence suggests that even lean, norm-glycemic individuals with a genetic predisposition to T2D such as those with diabetic parents often exhibit mild skeletal muscle insulin resistance. This underscores insulin resistance as an early contributor to T2D development. Skeletal muscle, as the primary site for insulin-stimulated glucose uptake, plays a critical role in whole-body insulin resistance. Studies show that when insulin resistance originates in skeletal muscle, addressing the issue in this tissue alone can restore systemic glucose homeostasis. While skeletal muscle insulin resistance is reversible through interventions like exercise and dietary modifications, β-cell dysfunction resulting from prolonged insulin resistance is irreversible to date. Insulin resistance affects not only the amount but also the timing of glucose uptake into skeletal muscle. Normally, postprandial glucose uptake in muscle increases steadily over time. However, in individuals with insulin resistance or T2D, delayed insulin action leads to a reduction in overall glucose uptake by skeletal muscle. This phenomenon has been observed in hyperinsulinemic-euglycemic clamp studies conducted on both diabetic and non-diabetic individuals. This highlights the critical role of skeletal muscle insulin resistance in the pathogenesis of T2D and the importance of early intervention to prevent disease progression (Merz and Thurmond 2020).

2.1.8 Insulin Receptor and Insulin Receptor Substrates: Key Players in Metabolic Regulation

Insulin signaling is central to various metabolic processes in skeletal muscle, including glucose transport, glycogen synthesis, and protein synthesis. The insulin receptor is a heterotetrametric glycoprotein composed of two α and two β subunits linked by disulfide bonds in an α-β-β-α configuration. The α subunit, located extracellular, serves as the insulin-binding domain, while the intracellular β subunits facilitate downstream signal transduction. When insulin binds to the α subunit, the receptor undergoes a conformational change, leading to tyrosine phosphorylation of the β subunit at the Tyr960 residue. This activation triggers the receptor's kinase activity, enabling the recruitment of insulin receptor substrates (IRSs). The IRS family consists of six proteins (IRS-1 to IRS-6), recruited to the insulin receptor via pleckstrin homology (PH) and phosphotyrosine binding (PTB) domains.

Tyrosine phosphorylation of IRS-1 by the insulin receptor is crucial for its activation. In skeletal muscle, IRS-1 plays a pivotal role in glucose uptake, as demonstrated in L6 myoblast studies, while IRS-2 appears to have a minimal role in this context, with its function in skeletal muscle still poorly understood. Interestingly, IRS-3 is present in rodent adipocytes, liver, and lung but is absent in humans. IRS-4 mRNA is expressed in several tissues, including skeletal muscle, liver, heart, brain, and kidney. IRS-5 and IRS-6 have limited expression across tissues and are relatively inefficient as insulin receptor substrates. This intricate network of insulin receptor and IRS proteins underscores their importance in skeletal muscle insulin signaling and glucose metabolism, highlighting their potential as therapeutic targets for managing insulin resistance and metabolic disorders.(Mann, Riddell, and Adegoke 2022)

2.1.9 IRS-1/PI3K/Akt Pathway in Muscle

The PI3K/Akt signaling pathway is a critical mechanism found across various organs, playing a pivotal role in numerous physiological processes. In insulin signaling, IRS-1 facilitates downstream signal propagation through PI3K to the Akt and extracellular signal-regulated kinase (ERK) pathways. Upon insulin binding to its receptor, the receptor undergoes phosphorylation, which subsequently phosphorylates tyrosine residues on IRS-1. This activation allows IRS-1 to interact with PI3K, a heterodimer composed of regulatory and catalytic domains. The regulatory domain connects to the tyrosine-phosphorylated IRS-1 via two Src homology 2 (SH2) domains, activating the catalytic domain. Once activated, the catalytic domain of PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to produce phosphatidylinositol (3,4,5)-triphosphate (PIP3), a lipid second messenger essential for downstream signaling. The phosphatase PTEN (phosphate and tensin homolog deleted on chromosome 10) acts as a negative regulator of this pathway.

PTEN dephosphorylates PIP3 back into PIP2, thereby preventing further insulin signaling. Mutations or loss of PTEN lead to hyperactivation of the PI3K signaling cascade, underscoring its importance in maintaining balanced insulin signaling. The formation of PIP3 activates 3-phosphoinositide-dependent protein kinase 1 (PDK1) by engaging its pleckstrin homology (PH) domain. PDK1 phosphorylates Akt and atypical protein kinase C (aPKC), initiating further downstream effects. Akt, in particular, phosphorylates the Akt substrate of 160 kDa (AS160), inhibiting Rab-GTPase activity and enabling Rab proteins to bind GTP. This process facilitates the translocation of GLUT4 to the plasma membrane, thereby promoting glucose uptake by the cell. Through the PI3K/Akt signaling pathway, insulin regulates key aspects of skeletal muscle metabolism, including glucose transport, glycogen synthesis, and protein synthesis. This pathway is fundamental to maintaining glucose homeostasis and is an essential target for understanding and treating insulin resistance and metabolic disorders. (Shanak et al. 2022; Mann, Riddell, and Adegoke 2022).

Insulin resistance in type 2 diabetes arises from disruptions at various points within the insulin signaling cascade in skeletal muscle. One of the hallmark abnormalities is the impairment of GLUT4 protein translocation to the cell membrane. This defect, driven by the inhibition of tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1), leads to reduced glucose uptake and is considered a primary metabolic disturbance in type 2 diabetes. Additionally, elevated activities of phosphatases such as protein tyrosine phosphatase 1B (PTP1B) and phosphatase and tensin homolog (PTEN) further exacerbate the condition by dephosphorylating key signaling molecules, thereby hindering insulin signaling (Al-Ishaq et al. 2019). A critical step in GLUT4 translocation is the insulin-stimulated phosphorylation of AS160, which has been shown to be diminished in patients with type 2 diabetes.

Research has also indicated that GLUT4 mRNA expression is reduced in individuals with type 2 diabetes due to defects in transcription and alterations in mRNA stability. These impairments contribute to the overall dysfunction in skeletal muscle glucose metabolism. Given the central role of skeletal muscle in glucose uptake, the insulin signaling pathway within this tissue has emerged as a crucial target for the prevention and management of type 2 diabetes. Advancing our understanding of this pathway is essential for the development of innovative therapies to treat diabetes and its related complications. (Rajendiran et al. 2024).

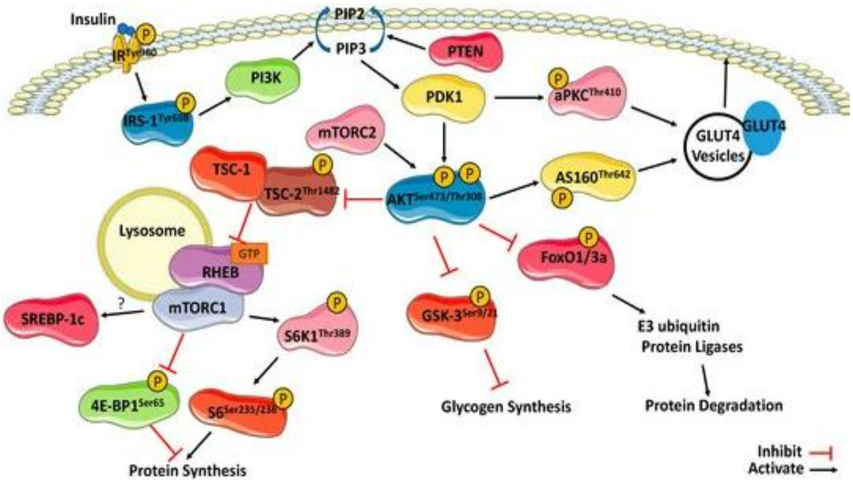

Figure 2.3: Simplified overview of insulin signaling in the skeletal muscle.(Mann, Riddell, and Adegoke 2022)

2.2 Medicinal plants with Anti-diabetic activities

The growing interest in herbal medicine over the past decade can be attributed to concerns over the long-term side effects of synthetic drugs, particularly those used for managing diabetes. Various classes of synthetic medications, such as biguanides, sulfonylureas, thiazolidinediones, α-glucosidase inhibitors, GLP-1 agonists, dopamine-2 agonists, DPP-4 inhibitors, and SGLT-2 inhibitors, are commonly prescribed. However, prolonged use of these drugs can lead to adverse effects, including cancer, liver damage, and allergic reactions. As a result, many individuals are increasingly turning to natural remedies, which are perceived to be less toxic than synthetic alternatives. The adage "Let food be the medicine, and medicine be the food" reflects the growing recognition that natural treatments offer numerous benefits, particularly due to their safety and minimal side effects(Ahda et al. 2023).

Natural plant extracts are gaining significant attention in the development of new therapeutic agents. Recent research highlights their effectiveness in treating or preventing a wide range of diseases, including cardiovascular conditions, atherosclerosis, diabetes, and cancer. Furthermore, herbal remedies have long been used globally for managing chronic illnesses(Owusu et al. 2020). Medicinal plants have emerged as a key area of interest, particularly for their potential in diabetes management. Over 410 medicinal plants have demonstrated experimentally proven anti-diabetic properties, offering therapeutic benefits without the harmful side effects often associated with conventional treatments.

These plants contain bioactive compounds such as tannins, alkaloids, flavonoids, and phenolics, which are essential for enhancing pancreatic function by regulating glucose absorption in the intestines and promoting insulin secretion. As such, medicinal plants present a promising and cost-effective alternative for diabetes treatment, making them an important focus in the search for alternative therapies for the condition. In developing countries, approximately 70–95% of the population relies on herbal medicinal plants for basic healthcare. The World Health Organization (WHO) estimates that around 65–80% of the global population depends primarily on herbal plants to treat various illnesses. This reliance is often due to limited access to modern medical facilities or the perception that herbal plants are a safer and more accessible source of healthcare(Huo and Qian 2018). Palestine, known for its rich biological diversity, boasts a wide range of traditional herbs used in local medicine. The region is home to over 2,600 plant species, with more than 700 identified as medicinal plants or used as botanical pesticides. A study by Ali-Shtayeh et al. (2000) found that at least 63 plant species are still in active use for treating a variety of ailments, including skin conditions, urinary tract issues, gastric problems, prostate diseases, diabetes and cancer. Further ethno pharmacological studies have highlighted the use of herbal medicine in Palestine for addressing gastrointestinal disorders, urological conditions, scalp diseases, and cancer treatment (Zaid et al. 2017).

2.2.1 Abelmoschus Esculentus

Abelmoschus esculentus (A.E), commonly known as okra shown in figure (2.4), is a member of the Malvaceae family, native to Africa but extensively cultivated and consumed in Asia, Southern Europe, and the Americas. Traditionally, okra has been used in folk medicine to enhance metabolic health, including in the management of type 2 diabetes mellitus (T2DM)(Nie et al. 2019; Zhang et al. 2020). The okra fruit contains proteins, fiber, polysaccharides, vitamins, and unsaturated fatty acids, as well as phenolic compounds such as polyphenols, terpenoids, alkaloids, and flavonoids(Sereno et al. 2022). These phytochemicals contribute to various health benefits, including antioxidant, anti-inflammatory, cardioprotective, gastroprotective, hepatoprotective, and particularly hypoglycemic effects, making okra both nutritionally and functionally valuable(Apolinário da Silva et al. 2024).

Figure 2.4: Abelmoschus Esculentus

2.2.2 Gundelia Tournefortii

Gundelia tournefortii (GT), a plant resembling artichoke, thrives in the semi-arid regions of the Mediterranean shown in figure (2.5). Commonly known as tumble thistle or Tournefort's gundelia, it belongs to the Asteraceae (Compositae) family and is a spiny perennial. As a wild edible, GT has demonstrated therapeutic effects against various conditions, including bacterial infections, cancer, epilepsy, gastrointestinal disorders, and diabetes. The anti-diabetic properties of GT were assessed in vivo using dexamethasone-induced diabetic mice, where oral administration of GT significantly lowered serum glucose levels. Additionally, GT extracts exhibited anti-diabetic activity in vitro by promoting GLUT4 translocation to the plasma membrane (Kadan et al. 2021a).

Figure 2.5: Gundelia Tournefortii

2.2.3 Hypericum Triquetrifolium

Hypericum Triquetrifolium (H.T) Turra (also known as H. crispum L.) is a flowering plant belonging to the Hypericaceae family, which consists of eight genera and approximately 600 species globally shown in figure (2.6). The genus Hypericum includes over 500 species of small trees, shrubs, and plants, with its largest subgenus also named Hypericum. Most species in this genus are native to warm-temperate regions and are found worldwide. H. triquetrifolium is native to northern Africa, western Asia, and southern and southwestern Europe, and it grows wild in Palestine. This plant has been shown to possess a wide range of pharmacological activities, including antioxidant, antibacterial, antinociceptive, anti-inflammatory, and cytotoxic properties. These effects are likely attributed to its rich content of secondary metabolites, such as flavonoids, naphthodianthrones, and phloroglucinols.(Qneibi et al. 2024)

Figure 2.6: Hypericum Triquetrifolium

2.2.4 Ocimum Bacillum

Ocimum Basilicum (O.B), commonly known as basil or Rehan, is an aromatic herb with a wide range of functional benefits as in figure (2.7). It has been traditionally used to treat conditions such as headaches, coughs, diarrhea, dyspepsia, flatulence, gastritis, constipation, warts, worms, and kidney malfunctions. In addition, it is a rich source of essential oils and aroma compounds, which contain biologically active constituents with insecticidal, nematicidal, fungistatic, and antimicrobial properties (Kadan et al. 2016). Due to its high content of phytochemicals, such as phenolic compounds and essential oils like estragol, linalool, and eugenol, Ocimum basilicum has been found to possess various pharmacological activities. These include blood glucose-lowering effects, antioxidant properties, anti-inflammatory actions, and potential in reducing the incidence of cancers and diabetes, as well as offering hepatoprotective benefits (Almalki 2019).

Figure 2.7: Ocimum Bacillum

2.2.5 Natural Plant-Based Medicine in Healthcare

Herbal medicine, a key component of complementary and alternative medicine (CAM), has been used for centuries to treat various illnesses and maintain health. CAM encompasses a diverse array of healthcare systems, practices, and products not typically considered part of conventional medicine

(AlQathama, 2016). According to the US National Center for Complementary and Alternative Medicine, herbal medicine involves the use of plant-derived products applied or consumed for therapeutic purposes. This ancient practice dates back over 5,000 years, yet scientific validation for its efficacy remains limited in many areas. Herbal medicines are widely utilized across healthcare systems, particularly in developing regions. The World Health Organization (WHO) estimates that approximately 80% of the population in developing countries, particularly in Africa and Asia, rely on herbal medicine for primary healthcare needs (WHO, 2019).

Furthermore, the popularity of herbal remedies has risen significantly in developed countries over the past two decades. For instance, 75% of the population in France and 42% in the United States report using herbal medicines, with similarly high usage observed in Middle Eastern nations such as Egypt (86%) and Saudi Arabia (88%) (AlQathama et al., 2020). This widespread adoption of herbal medicine has sparked considerable academic, industrial, and economic interest. Notably, chronic conditions such as diabetes mellitus, which are associated with high morbidity and mortality rates, have become a focus for integrating herbal remedies into therapeutic strategies. The global demand for herbal medicines underscores their potential role in modern healthcare while highlighting the need for further research to substantiate their safety and effectiveness (Zheng, Ley, and Hu 2018).

2.2.6 The Role of Medicinal Plants in Diabetes Management

Diabetes is a global health concern, with the World Health Organization (WHO) estimating that 347 million people worldwide were affected by the condition in 2013, a figure projected to double by 2030. More recently, WHO reported that 1.6 million deaths were directly attributable to diabetes, disproportionately affecting middle-income populations in Asia, Africa, and South America (Alqathama et al. 2020). Alarmingly, diabetes claims one life every six seconds, with annual death tolls reaching 5 million surpassing fatalities from HIV (1.5 million), tuberculosis (1.5 million), and malaria (0.6 million) combined. If not properly managed, diabetes can result in severe complications, including damage to vital organs such as the kidneys, heart, eyes, blood vessels, and nerves. The use of herbal remedies by diabetic patients is often motivated by dissatisfaction with conventional treatments and concerns about their potential side effects. Many patients perceive herbal medicines as safer and more effective alternatives due to their natural origins(Kesavadev et al. 2017)

Medicinal plants have been recognized for their significant role in managing diabetes mellitus, a serious metabolic disorder. These plants are reported to possess potent antidiabetic properties without harmful side effects. They contain bioactive compounds such as flavonoids, alkaloids, phenolics, and tannins, which enhance pancreatic function by promoting insulin secretion or reducing intestinal glucose absorption. Scientific literature has identified approximately 410 medicinal plants with experimentally validated antidiabetic properties, though the mechanisms of action have been comprehensively studied for only 109 of these plants. Plant extracts have demonstrated the ability to modulate critical metabolic pathways, including glycolysis, gluconeogenesis, the Krebs cycle, glycogen synthesis and degradation, insulin synthesis and release, cholesterol metabolism, and carbohydrate absorption (Bindu and Narendhirakannan 2019).

2.2.7 Phytochemicals

Phytochemicals are naturally occurring bioactive compounds found in plants that offer significant health benefits to humans. These compounds are categorized into primary metabolites, which include proteins, sugars, nucleic acids (pyrimidines and purines), amino acids, and chlorophylls, and secondary metabolites, such as flavonoids, alkaloids, saponins, phenolics, terpenes, curcumins, phytosterols, glucosides, lignans, and anthraquinones. Phytochemicals are found in a variety of plant-based sources, including fruits, vegetables, beverages, and cereals. They are primarily located in plant parts such as stems, roots, flowers, leaves, and seeds, often concentrated in the outer layers of these tissues. The concentration of phytochemicals varies depending on factors such as the plant species, growing conditions, and methods of processing or cooking. Given their diverse therapeutic properties, medicinal plants containing phytochemicals are invaluable in both complementing and potentially substituting modern medical treatments.(Sitobo, Navhaya, and Makhoba 2024)

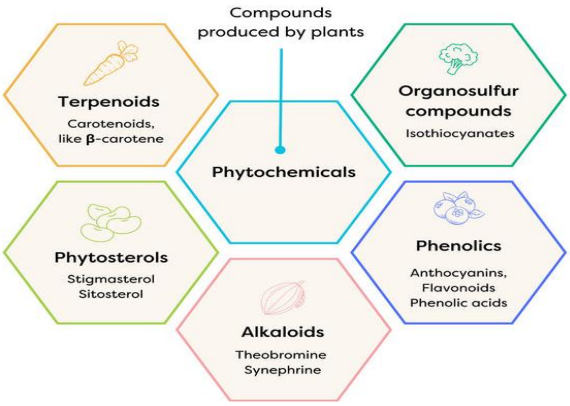

Figure 2.8: Phytochemicals in Plants Types (Ojo et al. 2023)

2.2.8 Phenolic Compounds in Medicinal Plants

Medicinal plants play a vital role in healthcare worldwide, serving as both standalone remedies and supplements to conventional treatments (Marmitt and Shahrajabian 2021). For centuries, plants have been utilized for food, flavoring, and medicinal purposes (Shahrajabian, Sun, and Cheng 2021). Various plant parts, possess diverse bioactive properties such as antioxidant and anti-inflammatory activities (Tirado-Kulieva, Hernández-Martínez, and Choque-Rivera 2022)

Among the most prevalent bioactive components are phenolic compounds, which include phenolic monoterpenes (e.g., carvacrol, thymol), diterpenes (e.g., carnosol, carnosic acid), and a range of phenolic acids like hydroxybenzoic acids (e.g., p-hydroxybenzoic, gallic, vanillic), phenylpropanoic acids (e.g., caffeic, rosmarinic, ferulic, chlorogenic), and flavonoids (e.g., naringenin, quercetin, catechin)(Sun, Shahrajabian, and Lin 2022). Plant phenolics have gained attention for their potential as effective antibiofilm and antifungal agents (Jaiswal and Kumar 2022).

Research has also shown a strong association between the levels of phenolic and flavonoid compounds and the anti-inflammatory and antioxidant properties of medicinal plants. Furthermore, studies indicate that the phenolic content of plants is closely linked to their antioxidant activity (Sun and Shahrajabian 2023), and are also considered precursors in the synthesis of pharmaceutical drugs used to treat a wide range of diseases, serving as crucial defense mechanisms to protect against harmful insects, pathogens, and adverse environmental conditions (Sitobo, Navhaya, and Makhoba 2024).

2.2.9 Anti-diabetic mechanism of natural phenolic compounds

Anti-diabetic mechanisms of natural phenolic compounds have gained significant attention due to their potential therapeutic effects. Non-flavonoid phenolics are primarily categorized into two basic carbon structures: hydroxycinnamic and hydroxybenzoic acids. These structures are further divided into four groups: phenolic acids, quinones, stilbenes, and coumarins. Research has shown that phenolic compounds not only possess antimicrobial properties but also offer significant nutritional benefits, including potential anti-diabetic effects. While some studies suggest that phenolics could help manage diabetes, further investigation, particularly with human trials, is necessary to substantiate these claims. More research is needed to understand the effects of pure phenolic compounds or phenolic-enriched diets in the context of diabetes management.

2.2.10 Chlorogenic acid

Chlorogenic acid (CA), also known as 5-caffeoylquinic acid (5-CQA), is a phenolic compound commonly found in plant-based human diets. It belongs to the hydroxycinnamic acid family and is formed through the combination of caffeic acid and quinic acid. Recent research has highlighted its wide range of Pharmacological properties, including hypoglycemic, hypolipidemic, anti-inflammatory, and antioxidant effects. Specifically,

CA has demonstrated the ability to alleviate and prevent diabetes mellitus (DM) and is beneficial in managing complications associated with DM, such as diabetic nephropathy (DN), diabetic retinopathy (DR), and diabetic peripheral neuropathy (DPN). CA has also been shown to improve insulin resistance, a primary cause of DM. (Yan et al. 2020) Studies indicate that chlorogenic acid enhances IRS-1-PI3K-Akt activation and promotes GLUT4 translocation in L6 myotubes. Chronic administration of CA to Lepr (db/db) mice improved insulin sensitivity, glucose tolerance, and lipid profiles, while reducing hepatic steatosis and increasing glucose uptake in skeletal muscles. Additionally, CA activates AMPK, contributing to beneficial metabolic effects such as reduced fatty acid synthesis and hepatic glucose production.(Shanak et al. 2022)

2.2.10 Caffeic acid

Caffeic acid (CA), also known as 3,4-dihydroxycinnamic acid, is a nonflavonoid phenolic acid naturally present in various herbs, foods, and beverages. As a potent antioxidant, CA helps reduce the formation of reactive oxygen species (ROS). It exerts anti-diabetic effects through multiple mechanisms, including the activation of antioxidant enzymes, inhibition of the nuclear transcription factor-κB (NF-κB) signaling pathway, and stimulation of the nuclear erythroid 2-related factor 2 (NrF2) transcription factor.(Akhlaghipour et al. 2023) Caffeic acid has been recognized for its dual role in regulating blood glucose levels, promoting insulin secretion from β cells while also helping to reduce insulin resistance, making it an effective agent in the management of diabetes mellitus (DM).(Salau et al. 2022).

2.2.11 Ferulic acid

Ferulic acid (FA), also known as (E)-3-(4-hydroxy-3-methoxy-phenyl) prop-2-enoic acid, is a phenolic compound commonly found in medicinal herbs and everyday foods. It has been demonstrated to possess several pharmacological effects, including antihyperglycemic, antihyperlipidemic, and antioxidant properties, which contribute to the management of diabetes and its complications. FA exhibits free radical scavenging activities, offering potential benefits in controlling cancer, cardiovascular diseases, as well as in hepatic protection, antimicrobial action, and anti-inflammatory treatments. In particular, its role in preventing and managing diabetes and related complications is well-documented. The antidiabetic effects of FA are linked to its ability to lower blood glucose levels, enhance antioxidant enzyme activity, and prevent lipid peroxidation in pancreatic tissues in diabetic rats. (Salau et al. 2023; Li et al. 2022).

2.2.12 Quercetin

Quercetin, a flavonoid, has been widely studied for its antidiabetic properties. Research indicates that quercetin improves endothelial function and enhances insulin production by up to 70% in isolated rat islets by modulating islet function. The suggested mechanisms include the alteration of calcium fluxes and cyclic nucleotide metabolism within the islet cells. Furthermore, quercetin promotes the regeneration of pancreatic islets, thus boosting insulin production. Quercetin is also known to influence several factors and signaling pathways involved in insulin resistance and the pathogenesis of type 2 diabetes, such as TNFα, NFKB, AMPK, AKT, and NRF2 (Yan et al. 2023; Chellian et al. 2022)

2.2.13 Vanillic acid

Vanillic acid (VA), a phenolic compound derived from vanillin, is commonly found in various fruits and vegetables. It is well-known for its potent antioxidant and antimicrobial properties, particularly its effectiveness against bacteria, molds, and yeasts. As an intermediate compound in vanillin biosynthesis, vanillic acid also exhibits pharmacological effects such as antimutagenic, antitumor, and anticlastogenic activities, positioning it as a promising nutraceutical agent. Its ability to address key aspects of diabetes pathogenesis—including oxidative stress, inflammation, and glucose metabolism—further underscores its therapeutic potential. Present in numerous antidiabetic plants, vanillic acid significantly contributes to their medicinal properties and stands out as a candidate for further research in diabetes treatment. Notably, studies suggest that vanillic acid may improve insulin signaling by enhancing the expression of GLUT4, a vital glucose transporter in muscle and fat tissues, thereby promoting efficient glucose uptake.(Singh et al. 2022; Ghasemzadeh Rahbardar, Ferns, and Ghayour Mobarhan 2025).

2.2.14 Benzoic Acid

Benzoic acid and its derivatives have garnered significant attention for their potential antioxidant and antidiabetic properties. These compounds exhibit remarkable antioxidant activity, effectively neutralizing free radicals and reducing oxidative stress. Evidence suggests that benzoic acid and its derivatives may provide therapeutic benefits in diabetes management by functioning as both antioxidants and enzyme inhibitors. Classified as a simple phenolic compound in plants, benzoic acid is characterized by its carboxyl group (-COOH) attached to a benzene ring, meeting the criteria for phenolic acids. In plants, benzoic acid and its derivatives play vital roles in various physiological processes and act as precursors for more complex phenolic compounds. They are widely recognized for their antioxidant, antimicrobial, and anti-inflammatory properties, contributing to the plant's defense systems. These attributes further underscore their potential health benefits, including their role in managing diabetes.(Deka, Choudhury, and Dey 2022)

The use of herbs as a potential alternative or complement to synthetic diabetes medications has garnered significant interest. This thesis aims to explore various herbal options for diabetes management, focusing on their active compounds, effectiveness, mechanisms of action, and safety profiles. These medicinal plants have been extensively utilized worldwide for diabetes treatment. The objective is to evaluate selected herbs as promising candidates for the development of future antidiabetic therapies.

Chapter Three: Methodology

Materials:

The plant materials used in this study were collected or purchased from various sources. Ocimum basilicum was purchased from Al Alim – Medicinal Herb Center, Zippori, Israel, while Gundelia tournefortii was collected from the northern Negev area in Israel. Abelmoschus esculentus was gathered from the hills of the West Bank during the spring and summer seasons, and Hypericum triquetrifolium was purchased from a medicinal plant's trader in Nablus, Palestine. For plant extract preparation, methanol (HPLC grade ≥99.8%) from Sigma-Aldrich, Germany, was used. L6-GLUT4myc cells, used for cell culture and treatment, were obtained from Kerafast, Boston, MA, USA, -MEM medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin from Biological Industries (Beit Haemek, Israel). For the MTT assay, MTT (Sigma-Aldrich, St. Louis, MO, USA) and for RNA extraction, TRIzol reagent (Thermo fisher Scientific), chloroform, isopropanol, and 75% ethanol from Sigma-Aldrich, Germany, High-capacity cDNA reverse transcription kit from Applied Biosystems™ (Life Technologies, Darmstadt, Germany). And the primers form Biotech. For analytical HPLC Acetonitrile (HPLC grade ≥99.9%) and phosphoric acid (prepared using orthophosphoric acid from Sigma-Aldrich, Germany) were used as mobile phases. Phenolic acids, such as Benzoic acid, Caffeic acid, Ferulic acid, Chlorogenic acid, Vanillin acid, and Quercetin acid from Sigma-Aldrich, Germany, were used as standards.

3.1. Plant collection

The aerial parts (stem, leaves and flowers) of Ocimum basilicum were purchased from an internationally recognized medicinal plants splaying company (Al Alim Medicinal Herb Center, Zippori, Israel), Gundelia tournefortii plant were collected from the northern Negev area in Israel, Abelmoschus Esculentus were collected from the hills of the West Bank during the spring and summer terms and Hypericum triquetrifolium were purchased from a medicinal plant's trader in Nablus, Palestine. The plants were identified by Prof. Nidal Jaradat (An-Najah

National University, Nablus, Palestine), cleaned and dried for 7 to 10 days in the shade at the obtained surrounding temperatures, then grounded and stored in cloth bags at 5°C. Then they were transferred to the laboratory for the preparation of plant extracts.

3.2. Plant Extract Preparation

The methanolic extract of *Ocimum Basilicum, Gundelia tournefortii, Abelmoschus esculentus and Hypericum triquetrifolium* were prepared as follows: Forty grams of air-dried aerial parts of each plant were powdered and packed in an Erlenmeyer flask with 200 mL methanol (HPLC grade ≥99.8%; Sigma Aldrich, Germany). The flasks were then sonicated for 2 h at 60 °C (Elmasonic, Singen, Germany) and left in dark glass bottles for 24 h for complete extraction. The yield of the extract in methanol were respectively 23.4%, 21.5%, 20.2% and 14.9%. The stock extracts were kept at −20°C in air-tight glass containers.(Bassalat et al. 2023)

3.3. L6 Cell Culture and Treatment

Rat L6 muscle cell line that were stably expressing myc-tagged GLUT4 (L6-GLUT4myc) obtained from Kerafast (Boston, MA, USA) and were cultured in a-MEM supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, and 0.1 mg/ml streptomycin (Biological Industries (Beit Haemek, Israel)) in an atmosphere of 95% air and 5% CO2. For every experiment, L6 cells that were approximately 80% confluent were collected. (Kadan et al. 2021b)

3.4. MTT Assay

The colorimetric tetrazolium dye MTT technique depends on the process by which mitochondrial succinate dehydrogenase in living cells changes the yellow tetrazolium bromide into its purple formazan derivative. In 96-microtiter plate wells, 20,000 cells were seeded. And incubated for 24 h, after that the cells were incubated with increasing concentrations of plant extract (0 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/mL) for 24 h at 37 °C. After that, the cells were maintained in serum-free RPMI after having been rinsed in phosphate-buffered saline. Next, 100 µL contained 0.5 mg/mL MTT (Sigma Aldrich (St. Louis, MO, USA)) were added to each 96well, and the plates were incubated for 4 hours. Following the removal of the media, the cells were treated with 100 µL of acidic isopropanol (0.08 N HCl) for 15 minutes in order to dissolve the formazan crystals. Using a microtiterplate reader, the MTT formazan's absorbance was measured at 570 nm.(Mahajna et al. 2019)

% Cell viability: $\frac{A570\, nm\, of\ plant\ extract\ treated\ sample}{A570\, nm\, of\ nontreated\ sample} \times 100$

3.5 L6 Myc Glut4 Plant Extract Treatment

80,000 L6-GLUT4 myc cells were seeded per each well of 6-microtiter plates and incubated for 24 h until the cells were confluent. Next, different plant methanolic extract groups (O. bacilicum, Gundelia tournefortii, Abelmoschus esculentus and Hypericum triquetrifolium) having a concentration of 125 µg /ml, and fraction isolated from A.E extract (1%) were added to the cells for 20 h, followed by serum starvation for 3 h and treated with and without 1 µM insulin for 20 min. The cells were washed twice with ice-cold PBS, Collected and stored at -80C. (Bassalat et al. 2023)

3.6 RNA Extraction

3.6.1 Lyse samples and separate phases

After relocating the treatment sample cells under the hood, 500 µl of TRizol reagent was added to each sample, which was then vortexed and placed on ice for a period of 10 minutes. Following a 5-minute centrifugation at 12,000 × g at 4◦C, the clear supernatant was transferred to a new tube and the process was repeated. The formed supernatant layer was then removed, and 200 µl of chloroform was added to each tube, thoroughly mixed by shaking, and incubated for 5 minutes to allow the nucleoprotein complex to completely dissociate. In order to improve separation of the mixture into a lower red phenol-chloroform layer, an interphase, and a colorless upper aqueous phase, the centrifugation period was extended to 15 min at 16,000 × g at 4◦C. (Zhang et al. 2023)

3.6.2 Isolate and precipitate RNA

To the aqueous phase, 250 µl of precooled isopropanol was added. It was then incubated for 15 minutes at 4°C to ensure the RNA precipitates sufficiently, centrifuged for 15 minutes at 12,000 × g at 4°C. Total RNA precipitate forms a white gel-like pellet at the bottom of the tube and the supernatant was discarded.(Zhang et al. 2023)

3.6.3 Wash and Solubilize RNA

After resuspending the pellets in 500 µl of 75% ethanol for every 500 µl of TRIzol lysis reagent, the sample underwent a brief vortex and was centrifuged for 5 minutes at 12,000 × g at 4°C. After using a pipettor to discard the supernatant, the RNA pellet was allowed to air dry for five to ten minutes. After adding 50 microliters of DEPC water to resuspend the pellet, the quality of each RNA extraction was verified using the Nanodrop Microvolume Spectrophotometer, which was based on the ratios of absorbance between A260 and A280. At -80°C, it was finally stored. (Zhang et al. 2023)

3.7 cDNA Synthesis

First-strand cDNA synthesis was performed via reverse transcription using the High-capacity cDNA reverse transcription kit (Applied Biosystems™, Life Technologies, Darmstadt, Germany). The amount of total RNA from each methanolic extract added to each cDNA reaction was always quantified using NanoDrop to reach 1000 ng. The RT master mix for each sample was prepared on ice as follow: 2 µl of 10× RT Buffer, 0.8 µl of 25× dNTP Mix (100Mm), 2 µl of 10x RT Random Primer, 1 µl Multi RT were added and completed to 10 µl with RNase Free Water then it was mixed gently. 10 µL of 2✕ RT master mix was mixed 10 µL of each RNA sample, mixed well by pipetting, tubes were then sealed and spin down. Samples were run with the PCR thermal cycling conditions as follows: 25◦C for 10minute, 37◦C for 120minute and 85◦C for 5minute. The generated cDNA samples were diluted ten-fold using RNAse-free water and used as a template for RT-PCR analysis.(Ostheim et al. 2022)

3.8 qReal time Polymerase reaction (qRT-PCR)

Syper Green Master Mix (Applied Biosystem, Thermo Fisher Scientific) was used to determine the mRNA expression for each cDNA sample, and the reactions contained 10µl of Syper Green Master Mix, 1µl of each primer (Biotech), 3µl of cDNA template, and RNase free water to a reaction volume of 20 µl. The cycling conditions was performed on a quantitative PCR machine (Bio Rad) as follow: a 10min initiation of Taq polymerase activity at 95 °C, 35 cycles of PCR amplification at 95 °C for 15 s, and a 1min annealing/elongation step at 60 °C. Primers sequences used were as follows:

Housekeeping Gene: **Rat 18S rRNA** F: 5’ GTAACCCGTTGAACCCCATT 3’R: 5’ CCATCCAATCGGTAGTAGCG 3’

Rat AS160 F: 5’-AGAAGGGGTCCCTAAAAGTCGGC-3’**,** R: 5’-GTTGGGCAATCTGTGTCTCAGGC-3’

Rat GLUT4 F: 5`- GTGTGGTCAATACCGTCTTCACG 3`, R: 5`-CCATTTTGCCCCTCAGTCATTC 3`

Rat IRS1 F: 5`ATGGCGAGAGCCCTCCGGATACC 3`**,** R:5`

CTCATAATACTCCAGGCGCGC 3`

Rat PTEN F: 5’-CAGAAGAAGCCCCGCCACCAG-3’R; 5’-AGAGGAGCAGCCGCAGAAATG-3’

Relative quantification results obtained for the target genes were normalized using the 2-ΔΔCT method. (Zhao et al. 2018)

3.9 Analytical HPLC

3.9.1 Chromatographic conditions

The HPLC analysis of the phenolic compounds and flavonoids was conducted using a Dionex HPLC system (thermofisher, germany), equipped with a UV-Vis DAD. The separation was conducted using a C18 reverse-phase chromatography column, 250 mm length, 4.6 mm width, and particle size 5 µm. The phenolic standard solutions and mixtures were injected into the system using an autoinjector, the gradient method was ultimately selected. It involves combining phosphoric acid (mobile phase B) and acetonitrile (mobile phase A, HPLC grade ≥99.9%; Sigma Aldrich, Germany). The phosphoric acid was prepared by adding 85% orthophosphoric acid (Sigma-Aldrich, Germany) dropwise to HPLC grade water (Sigma-Aldrich, Germany) until pH=2. The concentration gradient was changed in the following ways during the method's 60-minute total runtime: Initially, the ratio was 5% A to 95% B. After 15 minutes, it was 35% A to 65% B. After 20 minutes, it was 35% A to 65% B. At 30 minutes, it was 40% A to 60% B. After 35 minutes, it was 50% A to 50% B. g) 70% A and 30% B for 52 minutes; h) 5% A and 95% B for 60 minutes. There was a continuous flow rate of 0.5 mL/min and a 25 °C Column temperature. Following the examination of the different phenolic standards' UV-Vis spectra, three wavelengths: 210, 280, and 360 nm were selected for analysis in this study utilizing the HPLC-DAD.

3.9.2 Phenolics Standard Solution

From each of Phenolic acids (Sigma Aldrich, Germany): Benzoic acid, Caffiec acid, Ferrulic acid, chlorogenic acid, vanillin acid and quaracetin acid, 2mg was weighted and dissolved in 10ml methanol, sonicated for 5-10min, shacked and filter using 0.45µm filter to having a concentration of (0.2mg/ml).

3.9.3 Preparation of plant extract

From Abelmoschus Esculentus methanolic extract having a concentration of (40.4mg/ml), 5ml was diluted to 100ml with methanol, shacked and sonicated for 5min (2.02mg/ml). Then from that solution 1ml was diluted to 10ml with methanol to have a final concentration of (0.202mg/ml). (Mizzi et al. 2020).

3.10 Preparative HPLC analysis

3.10.1 Chromatographic conditions

The HPLC isolation and purification of the phenolic compounds were conducted using a ECOM Preparative HPLC system, The separation was conducted using a C18 reverse-phase chromatography column, 250 mm length, 20 mm width, and particle size 5 µm pore diameter 100A°.The gradient method that was eventually chosen following a series of preliminary studies uses a mixture of acetonitrile (mobile phase A, HPLC grade ≥99.9%; Honeywell Seelze, Germany) and phosphoric acid (mobile phase B), which was prepared by dropwise addition of 85% orthophosphoric acid (Sigma-Aldrich, Merck, Darmstadt, Germany) to HPLC grade water (Carlo Erba) until pH=2 was reached. The total runtime of the method was 60 min and the concentration gradient was varied as follows: a) initially 5% A and 95% B, b) 15 min 35% A and 65% B, c) 20 min 35% A and 65% B, d) 30 min 40% A and 60% B, e) 35 min 40% A and 60% B, f) 40 min 50% A and 50% B, g) 52 min 70% A and 30% B and h) 60 min 5% A and 95% B. A constant flow rate of 7.5 mL/min and a Column temperature of 25 °C were used. Following the analysis of the UV-Vis spectra of the individual phenolic standards, 280nm wavelength was chosen for the analysis in this investigation.

3.10.2 Preparation of plant extract

From Abelmoschus Esculentus methanolic extract having a concentration of (40.4mg/ml), 5ml was diluted to 100ml with methanol, sonicated for 5min, Shacked and filtered using 0.45µm filter membrane (2.02mg/ml) (Mizzi et al. 2020).

3.11 Statistical Analysis

Error limits were cited, and error bars were plotted and represent simple standard deviations of the mean. When comparing different samples, results were considered to be statistically different when $P < 0.05$. Student t-test was applied for statistical calculations using SPSS version 21.0 (Armonk, NY, USA). (Bassalat et al. 2023)

Chapter Four: Results

The aim of this study was to examine the mRNA expression levels of key proteins involved in the insulin signaling pathway, including IRS1, AS160, PTEN, and GLUT4, in L6 GLUT4 cells treated with plant extracts from Abelmoschus esculentus, Hypericum triquetrifolium, Ocimum basilicum, and Gundelia tournefortii, as well as the active compounds within specific extract. In summary, after treating L6 GLUT4 cells with these plant extracts, RNA was isolated, and cDNA synthesis was performed, followed by mRNA expression analysis using RT-PCR. The results demonstrated modulation of the expression levels of IRS1, AS160, PTEN, and GLUT4 in response to the treatments, indicating potential regulatory effects of these extracts on insulin signaling. Furthermore, the active compounds of Abelmoschus esculentus were identified, isolated, and purified using analytical and preparative high-performance liquid chromatography (HPLC). These isolated compounds are hypothesized to contribute significantly to the observed biological effects. Further characterization and validation of these compounds are ongoing to elucidate their precise role in modulating the insulin signaling pathway.

4.1 Biological Activity

4.1.1 MTT Cell Viability Test

To assess the potential cytotoxicity of the Abelmoschus esculentus, Hypericum triquetrifolium, Ocimum basilicum, and Gundelia tournefortii extracts on L6 GLUT4 cells, the MTT assay was utilized. This colorimetric assay measures cell metabolic activity by converting 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into insoluble formazan crystals via mitochondrial enzymes in viable cells. The cells were treated with plant extract concentrations of 125 µg/mL, 250 µg/mL, 500 µg/mL, and 1000 µg/mL and incubated for 24 hours. Following the incubation, the optical density of the dissolved formazan crystals was measured at 570 nm using a microplate reader, and the data recorded in table (4.1). No significant reduction in cell viability was seen after treatment with HT, G.T, O.B and A.E extracts up to 1000 µg/mL (Figure 4.1).

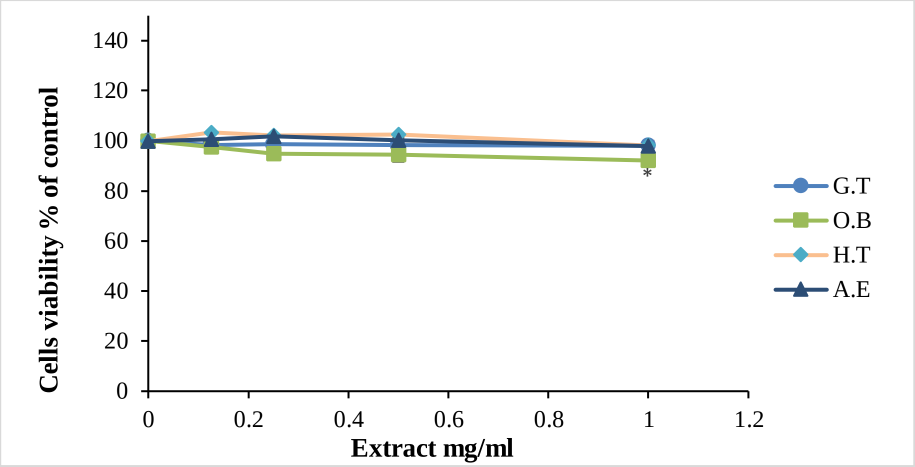

Figure 4.1 Effect of Abelmoschus esculentus, Hypericum triquetrifolium, Ocimum basilicum, and Gundelia tournefortii extract on cell viability examined by MTT assay. L6-GLUT4myc cells (20,000 cell/well) were seeded in 96-well plate and were exposed to increasing concentrations (0−1 mg/mL) extract for 24 h. Values given represent means ± SEM (% of untreated control cells) of three independent experiments carried out in triplicates.

4.2 RNA Concentration and Purity in A. esculentus and Its Phenolic Fractions under Insulin-Stimulated and Non-Stimulated Conditions

The concentration of RNA in the extracts varied as in Table (4.2), with the highest value observed in Abelmoschus esculentus (-Insulin) at 2365.8 ng/µL and the lowest in Hypericum triquetrifolium (-Insulin) at 241.4 ng/µL. The A260/A280 ratios for all samples were within the ideal range of approximately 1.8–2.0, indicating that the RNA is relatively pure with minimal protein contamination. Most samples also showed A260/A230 ratios above 1.2, suggesting good quality RNA. However, the Gundelia tournefortii (+Insulin) sample had a low A260/A230 ratio of 0.92, which could indicate possible contamination, such as from phenol, salts, or carbohydrates, which could affect RNA quality and qRT-PCR results, leading to inaccurate amplification. These samples would likely be re-extracted to ensure reliable data. In Table (4.3) below RNA Concentration: With the exception of Vanillic Acid and Chlorogenic Acid, where (+Insulin) is higher, (-Insulin) samples typically show higher RNA concentrations. A260/A280 Ratios: With only slight deviations, these ratios show stable RNA purity in relation to protein contamination in both (+Insulin) and (-Insulin) samples. A260/A230

Ratios: (+Insulin, -Insulin) samples typically have superior ratios, indicating less carbohydrate, phenol, or salt contamination.

4.3 q Real Time- Polymerase reaction (qRT -PCR)

4.3.1 Effect of Plant Extracts on GLUT4, PTEN, AS160, and IRS1 Expression in Glucose Metabolism Pathways

The GLUT4, PTEN, AS160, and IRS1 mRNA levels in L6 Myc Glut4 cells have been detected in this work using the reverse transcription-polymerase chain reaction (RT-PCR). Because RT-PCR is a reliable and sensitive method for identifying and quantifying gene expression, it is perfect for assessing small changes in mRNA levels in a controlled environment. Abelmoschus esculentus, Hypericum triquetrifolium, Ocimum basilicum, Gundelia tournefortii, and the active compounds in A.E. extracts were evaluated for their effects on GLUT4, PTEN, AS160, and IRS1 mRNA expression, normalized to 18S ribosomal RNA (18-sRNA) levels, which was chosen as the reference gene for skeletal muscle rat cells because it is a stable, abundant, and commonly used housekeeping gene in qRT-PCR experiments.

RNA from the (serum-starved and insulin-treated conditions) ensure that we were working with high-quality RNA, in this way validating housekeeping genes can ensure accurate normalization of gene expression in serum-starved or insulin-treated conditions. Additionally, the possible regulatory function of MYC was investigated in this context (Table 4.4).

As seen in figure (4.2), Hypericum triquetrifolium (HT) exhibits the strongest suppression of all three proteins, suggesting a significant effect on glucose transport and signaling pathways. All plant extracts (OB, AE, GT, and HT) significantly lower the expression of GLUT4, PTEN, and AS160 when compared to the control group. Significant inhibitory effects are also shown by Abelmoschus esculentus (AE) and Gundelia tournefortii (GT), indicating that they may affect glucose metabolism by focusing on these important proteins. Moderate regulation of glucose-related signaling pathways is shown by Ocimum basilicum's (OB) milder suppression of GLUT4, PTEN, and AS160. Unlike GLUT4, PTEN, and AS160, IRS1 expression varies between the plant extracts: OB exhibits an increase in IRS1 expression, whereas HT dramatically upregulates it, indicating a stimulatory impact.

While GT had no effect on IRS1 expression, suggesting selective regulation of signaling pathways, AE dramatically lowers IRS1 expression, suggesting an inhibitory role. In terms of biological sciences, OB may upregulate IRS1 and mildly decrease GLUT4, PTEN, and AS160, although its variability makes it less reliable. In addition to dramatically lowering IRS1 expression, AE also highly inhibits GLUT4, PTEN, and AS160, indicating a targeted inhibition of IRS1 signaling. GT exhibits selective regulation by strongly inhibiting GLUT4, PTEN, and AS160 while having no discernible impact on IRS1. The most powerful suppressor, HT, dramatically lowers the expression of GLUT4, PTEN, and AS160 while upregulating IRS1, demonstrating its dual function as a stimulator and a suppressor depending on the route.

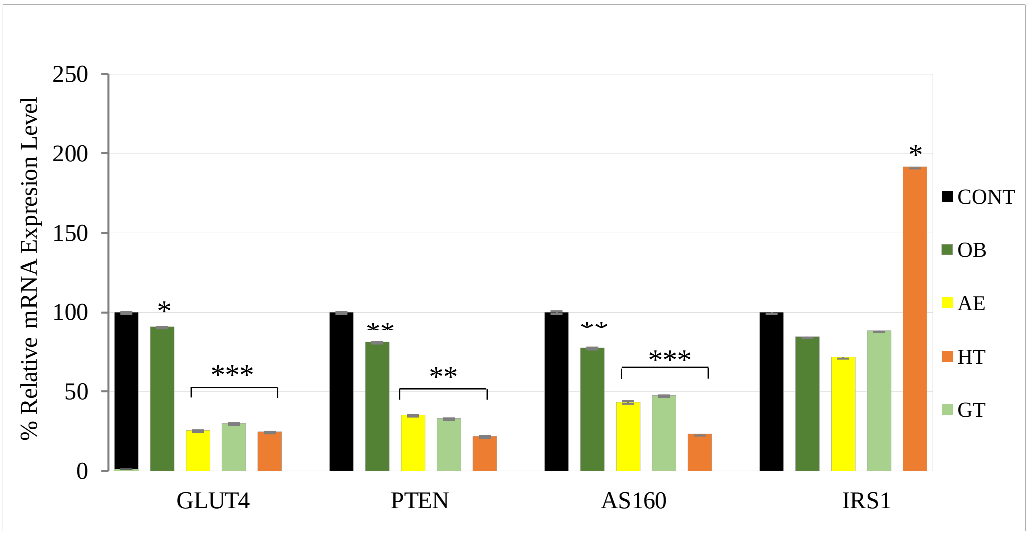

Figure 4.2: Effect of Abelmoschus esculentus, Hypericum triquetrifolium, Ocimum basilicum, Gundelia tournefortii methanolic Plant Extracts on GLUT4, PTEN, AS160, and IRS1 Expression in Glucose Metabolism Pathways

In conclusion, even though OB, AE, GT, and HT uniformly suppress the expression of GLUT4, PTEN, and AS160, but they have different effects on IRS1, which is indicative of their different functions in glucose metabolism and signaling. Also, both HT and AE affect insulin signaling in different ways, targeting different parts of the pathway This suggests that the final effect on insulin sensitivity might depend on which part of the pathway is more strongly influenced, and there might be a balance between increasing IRS1 (HT) and decreasing it (AE).

4.3.2 Impact of Plant Extracts on Insulin Signaling Markers in Serum-Starved L6 Myoblasts

Since natural plant extracts include bioactive chemicals that may improve glucose metabolism and insulin sensitivity, they have gained attention as possible medicinal agents. Using L6 myoblast cells under serum deprivation conditions with or without insulin therapy, this study examined the effects of methanolic extracts from four plants (OB, AE, GT, and HT) on the mRNA expression of important insulin signaling markers GLUT4, PTEN, AS160, and IRS1 (Table 4.5). Assessing whether these plant extracts alter insulin signaling and their capacity to replicate or intensify the effects of insulin was the goal. The findings reveal light on the ways in which these extracts affect important insulin signaling pathway markers, suggesting possible avenues for more research as natural treatments to enhance glucose metabolism.

4.3.2.1 GLUT4 mRNA Expression Analysis

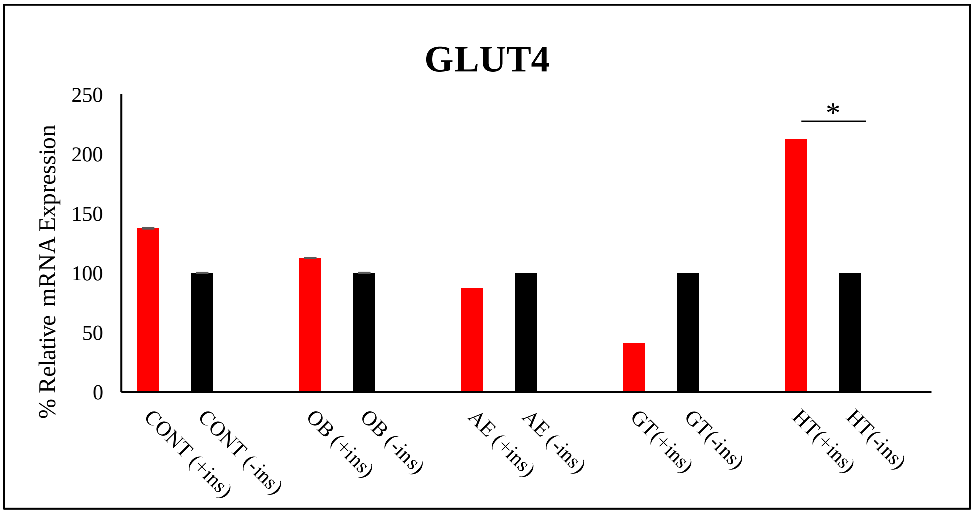

Figure 4.3: Effects of Plants Methanolic Extracts on GLUT4 gene expression before and after insulin stimulation

As seen in figure 4.3, the experiment's goal was to assess how a plant methanolic extract affected the expression of GLUT4 mRNA in L6 myoblast cells both with and without insulin administration. To normalize all samples and establish a common reference, the baseline (100% relative expression) was the insulin-free control condition (-insulin control). The insulin dependence of these extracts (OB, AE, GT, and HT) was then established by comparing +insulin to - insulin for each extract. GLUT4 expression rose to 137% when insulin was administered without any extract (CONT. + insulin) in comparison to the control, demonstrating that GLUT4 expression is upregulated by insulin stimulation.

However, when paired with insulin, the plant extracts showed different effects on GLUT4. The greatest increase in GLUT4 expression was observed in HT (+insulin) (212%, $p = 0.031$), indicating a considerable increase in GLUT4 mRNA levels and maybe better glucose uptake mechanisms, this increase in GLUT4 expression is significant and much larger than the 10–30% increase seen with thiazolidinediones (e.g., pioglitazone) (Which are a class of drugs used to improve insulin sensitivity, primarily in people with type 2 diabetes by Enhance insulin action in muscle and fat cells). This suggests HT may have a stronger impact on insulin sensitivity, though more studies are needed. However, GT (+insulin) caused GLUT4 expression to drop to 41% ($p = 0.075$, borderline significance), suggesting that GLUT4 expression may be inhibited even when insulin is stimulated. The GLUT4 expression levels from the other extracts, OB and AE (+insulin), were modest (112% and 87%, respectively), and the p-values (0.233 and 0.759) indicated that there was no statistically significant difference from the control. When the extracts were examined without insulin (CONT.- insulin), however, with values near 100%, GLUT4 expression stayed relatively stable despite every situation, suggesting that plant extracts by themselves have no noticeable impact on GLUT4 expression in the absence of insulin stimulation. These results imply that while some plant extracts, like GT, may block this pathway, others, like HT, may increase insulin-induced GLUT4 expression.

GLUT4 up regulation, do not always correlate with protein expression or functional glucose uptake, it’s important to confirm the functional relevance of the observed changes. In this case, assessing GLUT4 translocation to the cell surface was determine by Dr. Zaid's lab staff previously and to confirm the protein expression of GLUT4 and assess whether the mRNA up-regulation observed translates into an actual increase in GLUT4 protein levels western blot will be conducted.

4.3.2.2 PTEN mRNA Expression Analysis

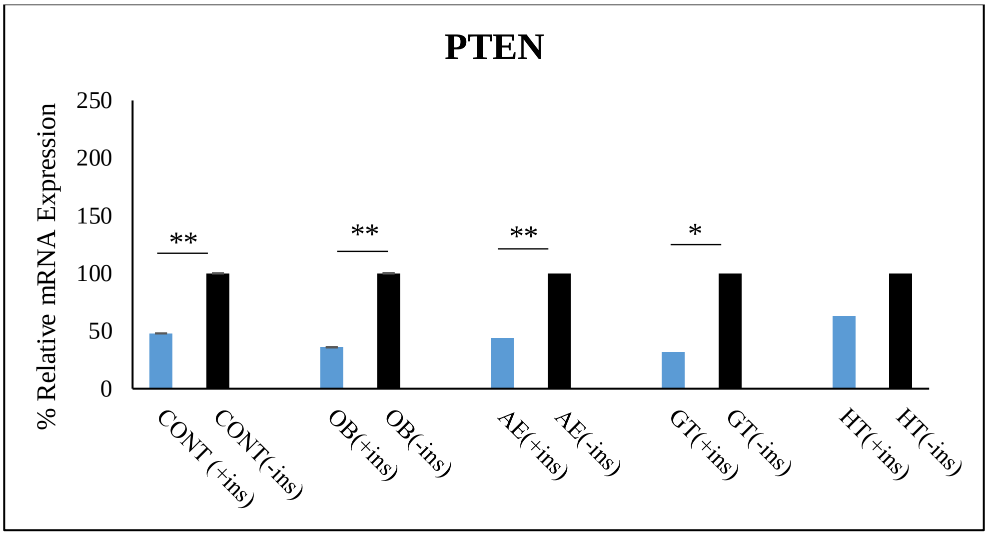

Figure 4.4: Effects of Plants Methanolic Extracts on PTEN gene expression before and after insulin stimulation

Figure 4.4: Displays the expression of PTEN: PTEN mRNA expression was also investigated in the experiment under various circumstances, both with and without insulin administration. To normalize all samples and establish a common reference, the baseline (100% relative expression) was the insulin-free control condition (-insulin control). The insulin-dependent PTEN levels altered by these extracts (OB, AE, GT, and HT) were then calculated by comparing +insulin to -insulin for each extract. PTEN expression dramatically dropped to 48% when insulin was administered without plant extract (CONT. + insulin) in comparison to the control ($p = 0.010$), demonstrating that insulin suppresses PTEN expression, a negative regulator of insulin signaling.

The findings were changed, though, when paired with plant extracts. PTEN expression was lowest in GT (+insulin) (32%, $p = 0.017$), indicating a significant downregulation that may improve insulin signaling. PTEN was also statistically significantly suppressed by OB (+insulin) and AE (+insulin), which decreased PTEN levels to 36% and 44%, respectively (p = 0.007 and 0.009). However, HT (+insulin) had a less statistically significant, more mild effect (63%, p = 0.078). On the other hand, PTEN expression remained high under all conditions when plant extracts were evaluated without insulin (CONT.EXT-insulin), with GT (-insulin) exhibiting the highest increase and OB (-insulin) following closely behind.

This suggests that in the absence of insulin, the extracts may possibly enhance PTEN expression rather than inhibit it. These findings imply that the plant extracts, specifically GT, OB, and AE, may enhance insulin signaling by significantly reducing PTEN expression when insulin is present. However, in the absence of insulin, these extracts appear to have the opposite effect, maintaining or increasing PTEN levels.

4.3.2.3 AS160 mRNA Expression Analysis

Expression for AS160 as seen in figure 4.5: Understanding the impact of plant methanolic extracts on insulin signaling is possible through the examination of AS160 mRNA expression under various circumstances. To normalize all samples and establish a common reference, the baseline (100% relative expression) was the insulin-free control condition (-insulin control). The insulin-dependent nature of these extracts was then ascertained by comparing +insulin to -insulin for each extract**.**

The data suggest that the control extract with insulin (CONT. +insulin) significantly suppresses AS160 expression, with only 15% expression relative to normal insulin activation. Among the tested extracts, AE effectively restores AS160 expression to 105%, indicating a strong insulin-dependent effect, though the change is not statistically significant (p = 0.366). OB, GT, and HT also increase AS160 expression but to a lesser extent. OB leads to a 26% expression increase with high significance (p = 0.005), while GT shows a moderate 27% increase but with very strong significance (p = 0.004). HT, with 47% expression, also demonstrates a statistically significant effect (p = 0.008). These results indicate that AE has the best potential for counteracting the extract-induced suppression of insulin signaling, while GT and HT may also have beneficial but weaker effects. Conversely, the control extract appears to impair AS160 activation, suggesting an inhibitory role in insulin signaling.

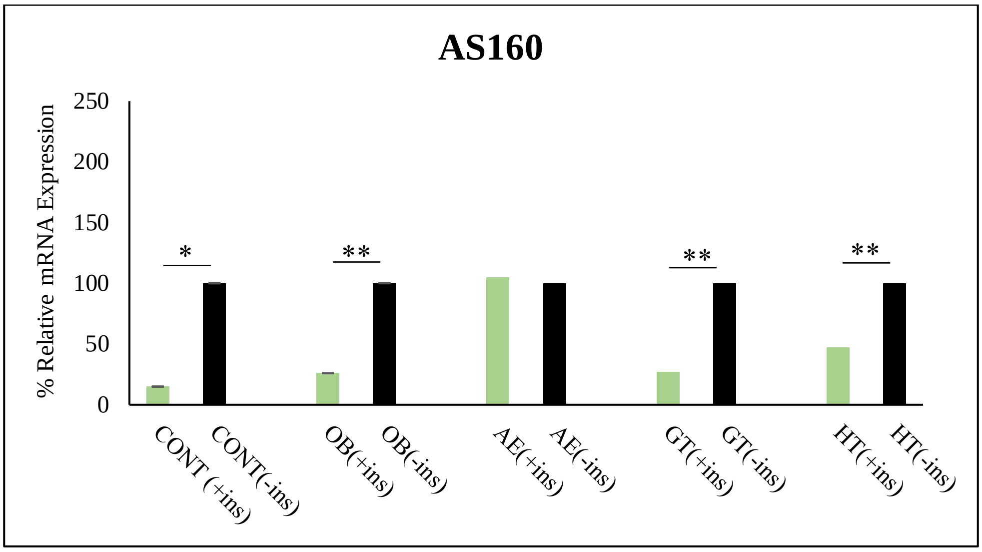

Figure 4.5: Effects of Plants Methanolic Extracts on AS160 gene expression before and after insulin stimulation

4.3.2.4 IRS1 mRNA Expression Analysis

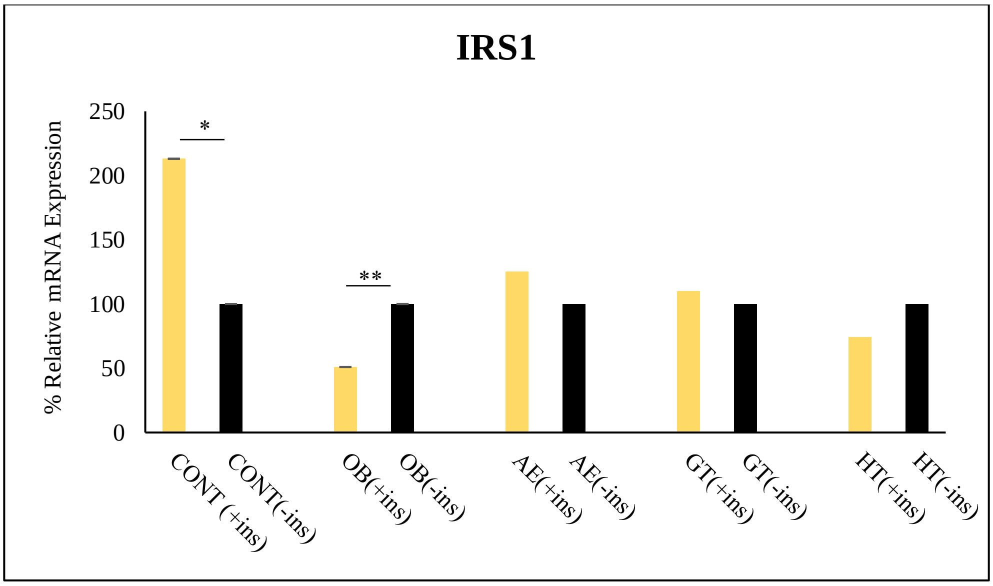

Figure 4.6: Effects of Plants Methanolic Extracts on IRS1 gene expression before and after insulin stimulation

Figure 4.6 displays the IRS1 expression: How the plant methanolic extracts affect insulin signaling is revealed by analyzing IRS1 mRNA expression under various circumstances. To normalize all samples and establish a common reference, the baseline (100% relative expression) was the insulin-free control condition (-insulin control). The insulin-dependent nature of these extracts was then ascertained by comparing +insulin to -insulin for each extract**.**

The results show that, in comparison to the studied extracts, the control extract containing insulin (CONT.EXT) results in a substantial IRS1 expression at 213%. Although AE has the greatest recovery impact of all the extracts, bringing IRS1 expression back to 125%, its non-significant p-value (0.390) indicates response variability. Additionally, although its impact is not statistically significant (p = 0.256), GT modestly raises IRS1 expression back to 110%. OB has a substantial suppressive impact, as evidenced by the dramatic reduction of IRS1 expression to 51% (p = 0.004). Although there is a fall in HT to 74%, it is not statistically significant (p = 0.224). According to these findings, AE appears to have the greatest capacity to restore IRS1 expression, but OB and HT seem to hinder insulin signaling by markedly lowering IRS1 activation.

4.4 Phenolic Profile of Abelmoschus esculentus methanolic Extract Analyzed by Analytical HPLC

Since HPLC can effectively identify key categories and representative phenolics despite the complexity and diversity of these compounds, it is frequently employed to investigate phenolic compounds in plants. The phenolic profile of the Abelmoschus esculentus (okra) extract was determined by HPLC analysis, and the results were quantified using standards, as shown in Tables (4.6). With an assay of 0.66%, caffeine acid is the most prevalent phenolic, highlighting the relative abundance of phenolics and suggesting a possible role in the extract's biological activity. Additionally, vanillic acid was significantly present (0.19%).

Despite being detected at lower amounts (Assay: 0.05%, 0.020%), chlorogenic acid and ferulic acid may still work in concert or separately to enhance the extract's effects. As shown in Figure (4.7), the other chemicals, benzoic acid and quercetin acid, exhibit moderate quantities, with assay values of 0.0535 and

0.0505 percent, respectively. Given that phenolics have well-established anti-inflammatory, antidiabetic, and antioxidant qualities, and since caffeine and other phenolics predominate, the extract is likely to have potent antioxidant and cell-protective effects. These results have important ramifications for studies investigating Abelmoschus esculentus's potential as a treatment for diseases like diabetes or oxidative stress-related

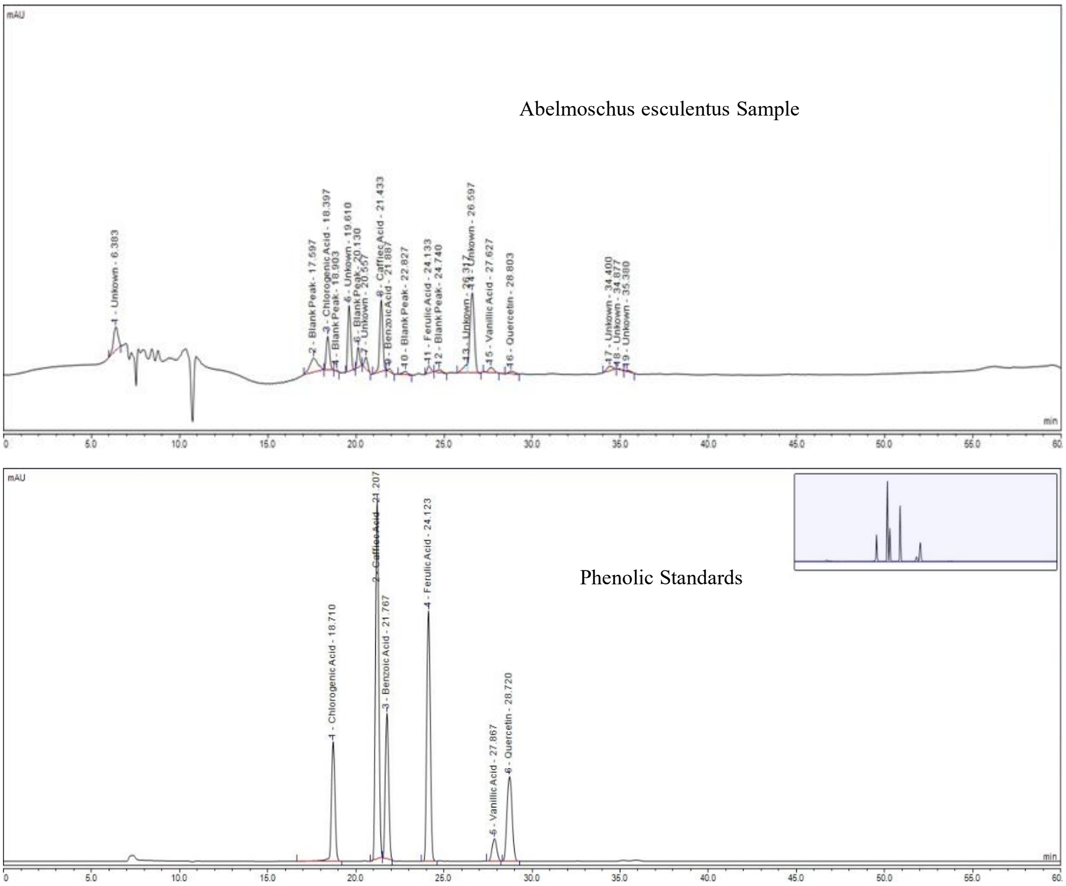

Figure 4.7: HPLC Analysis of Phenolic Compounds in Abelmoschus esculentus Extract

4.5 Isolation of Phenolic Compounds from *Abelmoschus esculentus* Using Preparative HPLC

One effective method for separating and purifying particular bioactive substances from various plant matrices is preparative high-performance liquid chromatography (HPLC). Key phenolic components discovered by first analytical HPLC profiling of Abelmoschus esculentus (okra) extract were isolated in this work using preparative HPLC, Figure (4.8). Caffeic acid, vanillic acid, chlorogenic acid, ferulic acid, benzoic acid, and quercetin acid are among the chemicals that are targeted; these were selected due to their biological significance and possible medicinal benefits, including antidiabetic, anti-inflammatory, and antioxidant activities. The purity of preparative HPLC fractions was validated using Analytical HPLC, which confirms purity by analyzing peak shape and area to ensure the presence of only the target compound. Residual solvents may interfere with downstream assays or biological activity. To ensure that these contaminants are minimized to maintain the accuracy and reliability of the results, we dried the fraction ten times using a water bath at 55°C.

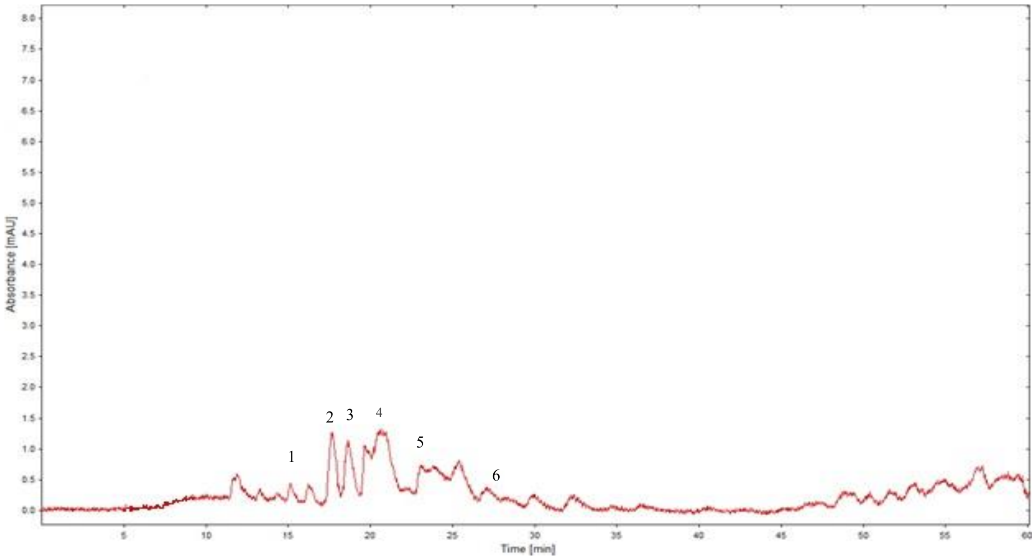

Figure 4.8: Isolation of Key Phenolic Compounds from Abelmoschus esculentus Extract by Preparative HPLC, Fraction represented as follow: **1:** Chlorogenic acid, **2:** Caffeic acid, **3:** Benzoic Acid, **4:** Ferrulic Acid, **5:** Vanillic Acid and **6:** Querecetine

An essential tool for examining their particular biological effects are the pure fractions that were acquired using preparative HPLC and dissolved in a solution of acetonitrile, water (PH=2.0), and methanol. Following solvent drying, these fractions were used to assess their effects on the mRNA expression levels of GLUT4, PTEN, AS160, and IRS1 in L6 Myc GLUT4 muscle cells at a concentration of 1% to ensure that this percent is non-toxic levels to avoid confounding effects on the results. These genes have a key role in insulin signaling, glucose metabolism, and cellular absorption of glucose. This work attempts to offer mechanistic insights into the function of individual phenolic compounds in signaling pathways by evaluating their impact on these markers. This strategy will help us better understand how Abelmoschus esculentus extract may be used therapeutically to treat metabolic diseases like diabetes.

4.6 Impact of Abelmoschus esculentus Fractions on insulin signaling Markers

4.6.1 GLUT4 mRNA Expression Analysis in Response to AE Extract and Phenolic Compounds

Important results are obtained from the examination of GLUT4 expression in (+insulin) conditions as seen in table (4.7), figure 4.9. With a significant p-value of 0.009 and a dependable GLUT4 expression of 66%, the control group exhibits a clear baseline level. With a p-value of 0.02 and a significant suppression effect, the solvent group exhibits a significant drop in GLUT4 expression to 14%, this reduction in GLUT4 expression should be accounted for by subtracting this effect from the GLUT4 expression observed with phenolic compounds, ensuring that any changes in GLUT4 are due to the solvent. Although the A.E. indicates that GLUT4 expression has increased to 114%, the p-value of 0.323 suggests that this effect is not statistically significant. With the highest relative expression (241%) and a significant p-value of 0.028, caffeine appears to considerably increase the expression of GLUT4.

In a comparable manner, chlorogenic acid exhibits a substantial effect with a p-value of 0.024 and a noteworthy rise of 168%. With a p-value of 0.183 and a relative expression of 140%, vanillic acid is not statistically significant. Ferulic acid exhibits a marginally significant rise (116%) with a p-value of 0.058. A highly significant p-value of 0.000 indicates quercetin's considerable inhibitory effect on GLUT4 expression, with a relative expression of 45%. Despite a 70% increase, benzoic acid's non-significant p-value of 0.349 indicates that it has no discernible impact on GLUT4 expression.

In conclusion, quercetin has a substantial inhibitory effect on GLUT4 expression, but caffeic acid and chlorogenic acid show the greatest promise. Other substances, such vanillic acid and ferulic acid, exhibit promise but need more research. Caffeic acid may contribute to the observed effects, but synergistic interactions with compounds like quercetin, which downregulates GLUT4, are likely influencing the overall outcome. Thus, the effects could result from both individual and combined actions of the compounds in AE.

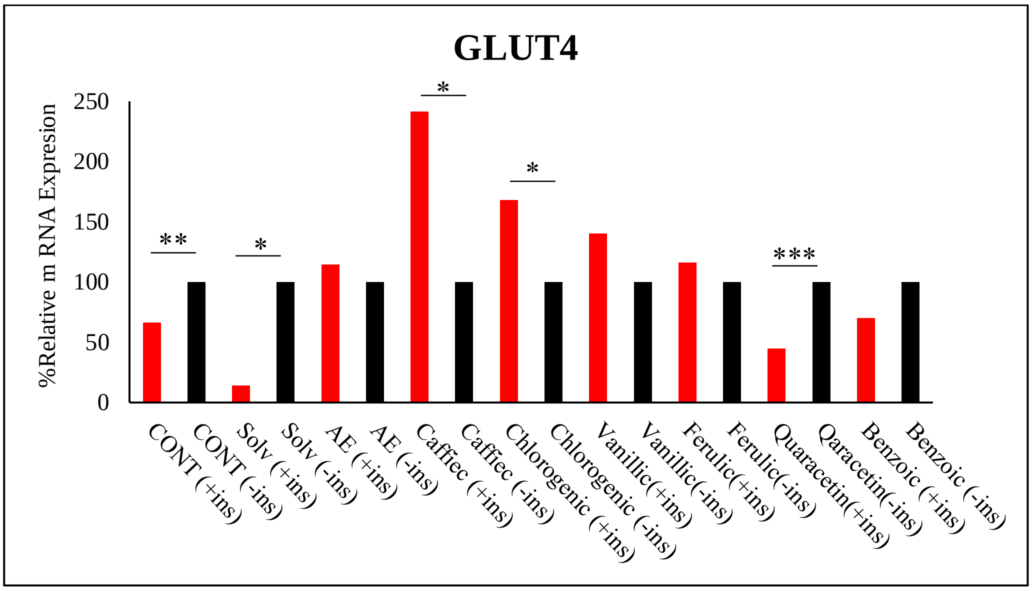

Figure 4.9: Effect of A. esculentus Extracts and it’s Phenolic Compounds on GLUT4 Expression in the Presence and Absence of Insulin

4.6.2 PTEN mRNA Expression Analysis in Response to AE Extract and Phenolic Compounds

Different phenolic substances have varied effects, according to the investigation of PTEN expression during insulin stimulation as seen in table (4.8), figure 4.10. Although the p-value shows that the effect is not statistically significant, chlorogenic acid shows the highest expression level (151%, $p = 0.221$), indicating a possible involvement in modifying PTEN expression. Although they have little statistical evidence, quercetin (117%, $p = 0.16$) and caffeic acid (96%, $p = 0.85$) also exhibit elevated expression. On the other hand, PTEN expression is dramatically decreased by benzoic acid (21%, $p = 0.003$) and vanillic acid (28%, $p = 0.033$), suggesting their potent inhibitory actions. There is no discernible effect of ferulic acid (0%, $p = 0.036$), indicating that it has no effect on PTEN expression under insulin circumstances. The solvent has a moderate but statistically significant effect, as seen by its 67% relative expression (p = 0.04).

Under insulin circumstances, the solvent, as a diluent, moderately influences PTEN expression (67%, p = 0.04), although its impact is negligible in comparison to active phenolic substances. All things considered, PTEN expression is marginally increased by chlorogenic acid, quercetin, and caffeic acid, considerably suppressed by vanillic and benzoic acids, and ineffective by ferulic acid.

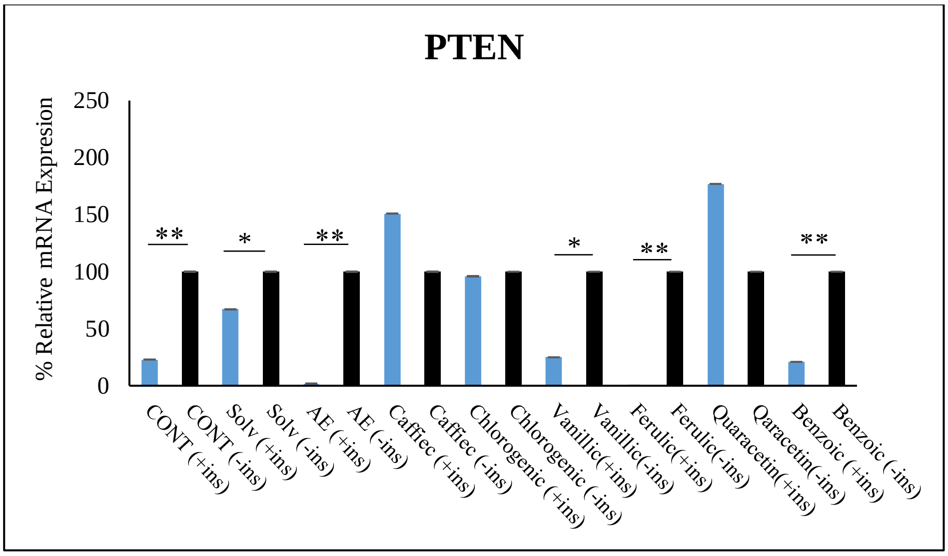

Figure 4.10: PTEN mRNA Expression Modulation by A. esculentus Methanolic Extracts and Phenolic Compounds

4.6.3 AS160 mRNA Expression Analysis in Response to AE Extract and Phenolic Compounds

Certain phenolic substances have a considerable impact on AS160 expression under insulin stimulation, whereas others have little to no effect, according to the research as seen in (Table 4.9), figure 4.11. Their substantial regulatory role in insulin response is demonstrated by the strongest effect of chlorogenic acid, which increases AS160 expression to 161% (p = 0.042), followed by benzoic acid at 138% (p = 0.021). Although both A.E. (126%, p =

0.074) and caffeine (167%, p = 0.138) exhibit elevated expression, their effects are not statistically significant. Vanillic acid is the least effective of the substances examined, with expression levels of quercetin (85%, p = 0.474), ferulic acid (120%, p = 0.194), and vanillic acid (30%, p = 0.034) being comparatively lower. The solvent raises the expression of AS160 to 171% (p = 0.062), although this effect is not statistically significant, suggesting a minor role in modulating AS160 expression.

Insulin moderately increases AS160 expression (171%) when the solvent is used as a diluent; nevertheless, the p-value (0.062) indicates that this impact is not statistically significant. In the presence of insulin, benzoic acid and chlorogenic acid generally have the greatest influence on AS160 expression, whereas quercetin, ferulic acid, and vanillic acid have less of an impact and the solvent has no effect.

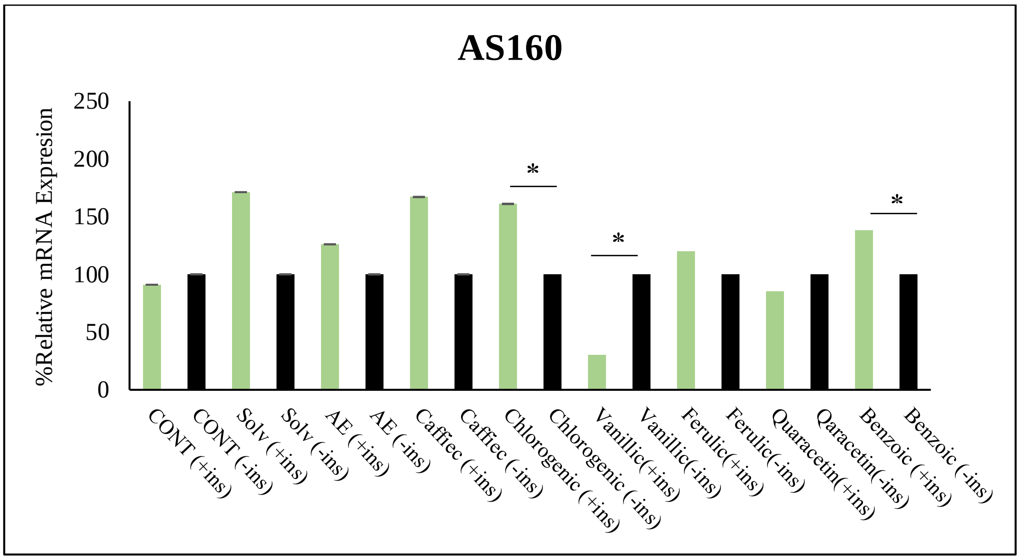

Figure 4.11**:** Effect of *A. esculentus* Methanolic Extracts and Phenolic Compounds on AS160 mRNA Expression in L6 Myotube Cells.

4.6.4 Effects of A. esculentus Extract and Phenolic Compounds on IRS1 Expression

Certain phenolic compounds dramatically increase IRS1 expression in the presence of insulin, whereas others have little to no effect, according to an investigation of IRS1 expression in response to various phenolic compounds.

With the greatest effect on IRS1 upregulation under insulin circumstances as shown in Table (4.10), figure 4.12, caffeine raises IRS1 expression to 372% (p = 0.013), followed by quercetin at 247% (p = 0.034) and chlorogenic acid at 228% (p = 0.036). The effects of ferulic acid (140%, p = 0.141) and A.E. (150%, p = 0.104) are not statistically significant, despite their moderate increases. Additionally, benzoic acid raises IRS1 expression to 247%, with a p-value of 0.034, which is borderline significant.

On the other hand, neither the solvent (59%, p = 0.122) nor vanillic acid (56%, p = 0.070) have any discernible impact, indicating that they are not significantly involved in IRS1 modulation in the presence of insulin. All treatments, including the solvent, retain 100% relative expression in the absence of insulin, suggesting that IRS1 regulation is not significantly affected. When insulin is present, the solvent, which is utilized as a diluent, marginally lowers IRS1 expression (59%), but this effect is not statistically significant (p = 0.122), and it has no effect when insulin is not present (100% relative expression). According to these results, the substances that increase IRS1 expression the most under insulin stimulation are caffeic acid, quercetin, and chlorogenic acid while vanillic acid, ferulic acid, and the solvent have very little effect.

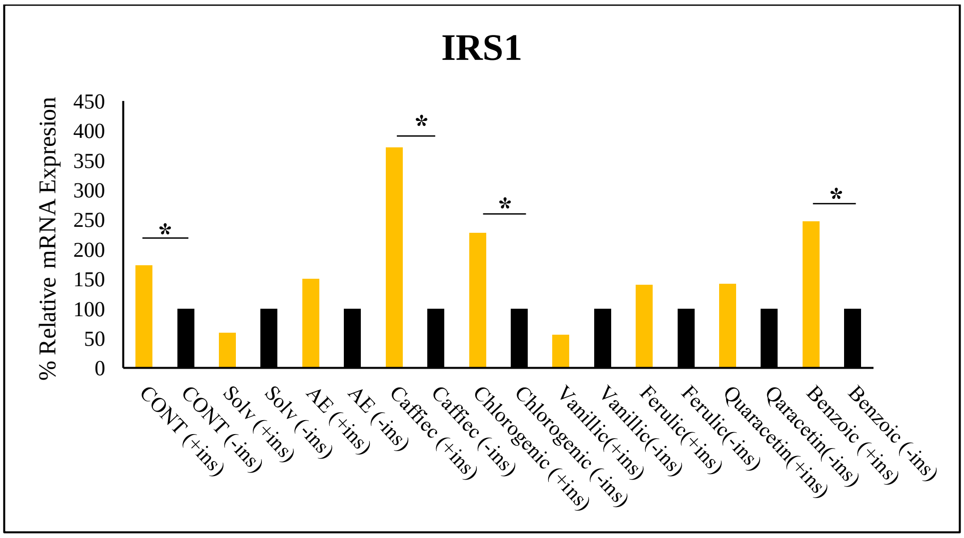

Figure 4.12: Effect of *A. esculentus* Methanolic Extracts and Phenolic Compounds on IRS1 mRNA Expression in L6 Myotube Cells

Chapter Five: Discussion

5.1 Restating research question and hypothesis:

The study's main hypothesis is that treating L6 GLUT4 cells with plant extracts from Abelmoschus esculentus, Hypericum triquetrifolium, Ocimum basilicum, and Gundelia tournefortii will alter the expression of important insulin signaling pathway proteins, including IRS1, AS160, PTEN, and GLUT4, which will increase GLUT4 translocation to the plasma membrane of skeletal muscle. According to the secondary hypothesis, the active ingredients in these plant extracts have a major role in the overexpression of these proteins, which enhances the skeletal muscle cells' ability to absorb glucose.

The following are the research questions that are driving this study:

1. What are the optimal concentrations of each plant extract that minimize cytotoxicity while preserving cellular viability?
2. What are the mRNA expression levels of key proteins involved in the insulin signaling pathway (e.g., IRS1, AS160, PTEN, and GLUT4) following treatment with antidiabetic plant extracts (*Abelmoschus esculentus*, *Hypericum triquetrifolium*, *Ocimum basilicum*, and *Gundelia tournefortii*), and how do these levels influence GLUT4 translocation to the skeletal muscle plasma membrane?
3. Which active compounds within *Abelmoschus esculentus* extracts demonstrate the highest antidiabetic activity, contributing most effectively to glucose uptake in skeletal muscle cells and their mRNA expression levels?

5.2 Summary of main findings:

In L6 GLUT4 muscle cells, the study assessed the cytotoxicity and potential for treatment of extracts from Abelmoschus esculentus (A.E.), Hypericum triquetrifolium (H.T.), Ocimum basilicum (O.B.), and Gundelia tournefortii (G.T.). Cell viability for further efficacy tests at concentrations up to 1000 µg/mL was ensured by the MTT assay, which verified that all extracts were non-toxic up to that point. A.E. extract's HPLC examination revealed a rich phenolic profile, with the most prevalent phenolic acid (0.66%) being caffeine, followed by vanillic acid (0.19%), and modest levels of chlorogenic acid, ferulic acid, benzoic acid, and quercetin acid. These phenolics emphasize the extract's possible biological activity because of their anti-inflammatory, antidiabetic, and antioxidant qualities.

Methanolic extracts from four plant species—OB, AE, GT, and HT—were used in this study to examine the effects of insulin administration and non-treatment on important insulin signaling markers (GLUT4, PTEN, AS160, and IRS1) in L6 myoblast cells. The findings demonstrated that although GT decreased GLUT4 expression, pointing to a potential inhibitory effect, HT significantly increased GLUT4 expression ($p < 0.05$) showing a potential to promote glucose absorption. In the presence of insulin, the majority of extracts downregulated PTEN expression; notable decreases were seen in GT, OB, and AE ($p < 0.05$), which may improve insulin signaling. Additionally, AE successfully restored the expression of AS160 and IRS1, indicating that it could aid in the fight against insulin resistance.

HT’s strong suppression of GLUT4, PTEN, and AS160, alongside IRS1 upregulation, suggests it may act through multiple pathways, potentially influencing both AMPK and PI3K signaling. The suppression of PTEN (a PI3K inhibitor) and AS160 (a downstream Akt target) hints at enhanced PI3K/Akt signaling, while GLUT4 suppression raises questions about its overall metabolic impact. Compared to other Hypericum species, variations in bioactive compounds may lead to different effects on insulin signaling, warranting further studies to clarify whether HT’s mechanism is unique or aligns with known antidiabetic properties of related species.

The need for insulin co-treatment to modulate GLUT4 suggests that plant extracts like AE may enhance insulin sensitivity rather than act as insulin substitutes. If AE alone does not significantly increase GLUT4 but amplifies insulin's effect (87% vs. ~100%), it likely works by improving insulin signaling rather than directly mimicking insulin. This aligns with mechanisms seen in insulin-sensitizing compounds like metformin, which enhance insulin action rather than replace it.

The main phenolic components in AE that were found to be responsible for its insulin-sensitizing actions were caffeic and chlorogenic acids. On the other hand, Quercetin’s suppression of GLUT4 (45%) but upregulation of IRS1 (247%) presents a complex effect on insulin signaling. Prior studies suggest that quercetin enhances insulin sensitivity, primarily by modulating IRS1/PI3K/Akt signaling, which aligns with its observed IRS1 upregulation. However, its GLUT4 suppression contrasts with reports showing GLUT4 upregulation in some models, suggesting a cell-type-specific effect. Differences in cell type, dose, duration, or metabolic state may explain these variations, highlighting the need for further investigation into its precise mechanism across different models. All things considered, the study demonstrates how plant extracts, particularly HT and AE, might improve insulin signaling. Among them, AE has the most promising effects on glucose metabolism by modulating important insulin signaling markers. These findings suggest that certain plant extracts, particularly HT and AE, may have potential benefits in modulating insulin signaling pathways, warranting further investigation in preclinical and clinical settings.

5.2 Research Significance and Contribution:

This study enhances our understanding of plant-derived compounds in modulating insulin signaling, particularly in skeletal muscle glucose uptake. Examining how extracts from Abelmoschus esculentus, Hypericum triquetrifolium, Ocimum basilicum, and Gundelia tournefortii affect the expression of key insulin signaling proteins, including IRS1, AS160, PTEN, and GLUT4, is the goal of the study. By examining these relationships, this study may help create new, plant-based treatment approaches that enhance insulin sensitivity and glucose metabolism, especially in skeletal muscle, a vital organ for maintaining glucose homeostasis. Additionally, the discovery of active substances in these plant extracts that improve insulin signaling and encourage glucose uptake may provide fresh understanding of the molecular underpinnings underlying antidiabetic effects.

This study may lead to the creation of complementary or alternative diabetic treatments, particularly in areas where access to traditional medications may be restricted or where people prefer natural cures. In the end, this research will add to the increasing amount of data sensitivity and control blood sugar levels in people with diabetes or at risk of getting the condition. Demonstrating the effectiveness of medicinal plants in treating metabolic diseases and could provide a viable path for upcoming therapeutic interventions meant to enhance insulin.

5.3 Future Research Directions:

Future studies in this area could build on the findings by examining a number of important areas. In order to comprehend the molecular pathways and post-translational modifications involved in the modulation of insulin signaling proteins by plant extracts, more thorough mechanistic research is first required. In order to evaluate the pharmacokinetics and therapeutic potential of these extracts' active ingredients and validate their safety and effectiveness in animal models or clinical trials, in vivo investigations are also essential and this can be done by collaborate with pharmacologists and clinical researchers. A more focused method of comprehending the plants' effects on insulin signaling and glucose metabolism may be possible through the isolation and identification of their bioactive components. Furthermore, investigating the synergistic effects of various extracts or chemicals may result in more effective diabetes treatment compositions. To improve dosage schedules and prevent negative reactions, it is crucial to confirm the safety of these extracts by cytotoxicity and safety profile investigations. Finally, converting these results into clinical trials can help assess how well these plant extracts work to control insulin resistance and complications associated with diabetes, ultimately assisting in the creation of innovative and easily accessible diabetes treatment methods. Human Induced Pluripotent Stem Cells (iPSCs), which can differentiate into muscle cells that more closely resemble human skeletal muscle, are also useful for finding more pertinent experimental models for translational studies to better imitate human physiology. Additionally, they can be genetically altered to investigate medicinal approaches or disease models.

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Appendices

Table 4.1: Abelmoschus esculentus, Hypericum triquetrifolium, Ocimum basilicum, and Gundelia tournefortii extract's % cell viability as determined by the MTT assay

G. Tournefortia	**Control**		**Concentration (mg/ml)**			
Plant conc. (mg/ml)	0		0.125	0.25	0.5	1
Absorbance	0.749	0.785	0.734	0.743	0.739	0.739
	0.739	0.775	0.732	0.743	0.714	0.707
	0.773	0.76	0.793	0.768	0.773	0.799
	0.77	0.787	0.764	0.775	0.788	0.763
Average	0.767		0.756	0.757	0.754	0.752
%Cell Viability	100		98.5	98.7	98.2	98.0
O. Basilicum	**Control**		**Concentration (mg/ml)**			
Plant conc. (mg/ml)	0		0.125	0.25	0.5	1
Absorbance	0.749	0.785	0.723	0.723	0.718	0.692
	0.739	0.775	0.694	0.699	0.62	0.611
	0.773	0.76	0.793	0.706	0.803	0.807
	0.77	0.787	0.781	0.782	0.763	0.718
Average	0.767		0.748	0.728	0.726	0.707
%Cell Viability	100		97.5	94.8	94.6	92.1
H. Triquetrifolium	**Control**		**Concentration (mg/ml)**			
Plant conc. (mg/ml)	0		0.125	0.25	0.5	1
Absorbance	0.749	0.785	0.813	0.782	0.768	0.728
	0.739	0.775	0.788	0.777	0.767	0.755
	0.773	0.76	0.791	0.801	0.82	0.768
	0.77	0.787	0.781	0.78	0.792	0.761
Average	0.767		0.793	0.785	0.787	0.753
%Cell Viability	100		103.4	102.3	102.5	98.1
A. Esculentus	**Control**		**Concentration (mg/ml)**			
Plant conc. (mg/ml)	0		0.125	0.25	0.5	1
Absorbance	0.749	0.785	0.769	0.789	0.749	0.752
	0.739	0.775	0.75	0.777	0.761	0.746
	0.773	0.76	0.771	0.761	0.748	0.724
	0.77	0.787	0.802	0.798	0.816	0.783
Average	0.767		0.773	0.781	0.769	0.75125
%Cell Viability	100		100.7	101.8	100.2	97.9

Table 4.2: RNA Concentration and Purity in A. esculentus, H. triquetrifolium, G. tournefortii and O. basilicum under Insulin-Stimulated and Non-Stimulated Conditions

No.	**Sample**	**ng/µL**	**A260/A280**	**A260/A230**
1	Control (+Insulin)	1304	1.92	1.21
2	Control (-Insulin)	1000.9	1.93	1.3
3	Hypericum triquetrifolium (+Insulin)	729.2	1.91	1.24
4	Hypericum triquetrifolium (-Insulin)	241.4	1.94	1.31
5	Gundelia tournefortii (+Insulin)	792.9	1.97	0.92
6	Gundelia tournefortii (-Insulin)	297.6	1.89	1.2
7	Ocimum basilicum (+Insulin)	398.7	1.92	1.29
8	Ocimum basilicum (-Insulin)	1024.9	1.94	1.31
9	Abelmoschus esculentus (+Insulin)	1217.5	1.91	1.24
10	Abelmoschus esculentus (-Insulin)	2365.8	1.92	1.29

Table 4.3: RNA Concentration and Purity in A. esculentus and Its Phenolic Fractions under Insulin-Stimulated and Non-Stimulated Conditions

No.	**Sample**	**ng/ µL**	**A260/A280**	**A260/A230**
1	Abelmoschus esculentus (+Insulin)	546.7	1.9	0.71
2	Abelmoschus esculentus (-Insulin)	542.8	1.88	0.69
3	Solvent (+Insulin)	359.6	1.91	1.24
4	Solvent (-Insulin)	379.5	1.89	1.2
5	Control (+Insulin)	463.7	1.87	0.7
6	Control (-Insulin)	536.9	1.9	0.69
7	Vanillic Acid (+Insulin)	380.8	1.91	0.66
8	Vanillic Acid (-Insulin)	318.5	1.89	1.2
9	Benzoic Acid (+Insulin)	476.9	1.9	0.77
10	Benzoic Acid (-Insulin)	483.2	1.89	0.72
11	Ferrulic Acid (+Insulin)	388	1.88	0.69
12	Ferrulic Acid (-Insulin)	489.3	1.88	0.69
13	Quaracetin (+Insulin)	360.4	1.86	0.7
14	Quaracetin (-Insulin)	464.1	1.85	0.69
15	Caffiec Acid (+Insulin)	394.5	1.93	0.79
16	Caffiec Acid (-Insulin)	478.8	1.94	0.74
17	Chlorogenic Acid (+Insulin)	409.4	1.86	0.66
18	Chlorogenic Acid (-Insulin)	323.6	1.9	0.75

Table 4.4: Effect of Plant Extracts on GLUT4, PTEN, AS160, and IRS1 Expression in Glucose Metabolism Pathways

GLUT4	**Cont.**	**OB**	**AE**	**GT**	**HT**
Sample Rel. Expression	0.95	0.93	0.27	0.32	0.22
	1.01	0.9	0.26	0.29	0.25
	1.05	0.9	0.24	0.3	0.27
Mean	1	0.91	0.26	0.3	0.25
SD	0.05	0.02	0.01	0.02	0.03
SE	0.03	0.01	0.01	0.01	0.02
p-value	-	0.04	0	0	0
%Rel. Expression	**100**	**91**	**26**	**30**	**25**
PTEN	Cont.	OB	AE	GT	HT
Sample Rel. Expression	0.95	0.76	0.33	0.3	0.18
	1.06	0.87	0.35	0.35	0.24
	1	0.81	0.37	0.35	0.24
Mean	1	0.81	0.35	0.33	0.22
SD	0.06	0.05	0.02	0.03	0.04
SE	0.03	0.03	0.01	0.02	0.02
p-value		0.01	0	0	0
%Rel. Expression	**100**	**81**	**35**	**33**	**22**
AS160	Cont.	OB	AE	GT	HT
Sample Rel. Expression	1.06	0.81	0.48	0.44	0.24
	1.03	0.76	0.43	0.51	0.22
	0.92	0.76	0.39	0.49	0.24
Mean	1	0.78	0.43	0.48	0.23
SD	0.07	0.03	0.04	0.04	0.01
SE	0.04	0.02	0.03	0.02	0.01
p-value	-	0.01	0	0	0
%Rel. Expression	**100**	**78**	**43**	**48**	**23**
IRS1	Cont.	OB	AE	GT	HT
Sample Rel. Expression	0.96	1.1	0.74	0.83	1.7
	1.4	0.99	0.7	1.18	2.42
	0.98	0.98	0.79	0.9	1.83
Mean	1.1	1.02	0.7	0.97	2
SD	0.25	0.06	0.04	0.19	0.38
SE	0.14	0.04	0.03	0.11	0.22
p-value	-	0.57	0.06	0.46	0.03
%Rel. Expression	**100**	**92**	**67**	**87**	**178**

Table 4.5: Effect of A. esculentus Extracts and it Phenolic Compounds on GLUT4, PTEN, AS160 and IRS1 Expression in the Presence and Absence of Insulin

GLUT4	**Cont. (+ins)**	**Cont. (-ins)**	**OB (+ins)**	**OB (-ins)**	**AE (+ins)**	**AE (-ins)**	**GT (+ins)**	**GT (-ins)**	**HT (+ins)**	**HT (-ins)**
Rel. Exp.	1.45	1.13	0.261	0.212	0.14	0.10	0.11	0.35	0.44	0.15
	1.43	1.21	0.207	0.209	0.11	0.24	0.12	0.20	0.48	0.27
	1.34	0.73	0.221	0.192	0.12	0.09	0.14	0.36	0.33	0.17
Mean	1.4	1.0	0.229	0.204	0.12	0.14	0.13	0.30	0.42	0.20
SD	0.057	0.256	0.028	0.010	0.014	0.081	0.017	0.092	0.079	0.065
SE	0.033	0.148	0.016	0.006	0.008	0.047	0.010	0.053	0.046	0.038
P-Value	**0.084**		**0.233**		**0.759**		**0.075**		**0.031**	
%mRNA Expression	**137**	**100**	**112**	**100**	**87**	**100**	**41**	**100**	**212**	**100**
PTEN Rel. Exp.	0.46	1.06	2.538	5.701	0.59	1.14	3.10	8.08	0.66	1.76
	0.49	0.91	1.870	5.921	0.28	1.03	2.85	10.81	1.51	1.96
	0.49	1.04	2.077	6.197	0.59	1.19	3.11	9.65	1.36	1.88
Mean	0.5	1.0	2.162	5.940	0.49	1.12	3.02	9.51	1.17	1.87
SD	0.017	0.077	0.342	0.249	0.179	0.081	0.147	1.370	0.453	0.101
SE	0.010	0.045	0.198	0.144	0.103	0.046	0.085	0.791	0.262	0.058
P-Value	0.010		0.007		0.009		0.017		0.078	
%mRNA Expression	**48**	**100**	**36**	**100**	**44**	**100**	**32**	**100**	**36**	**100**

Table 4.5: Effect of A. esculentus Extracts and it Phenolic Compounds on GLUT4, PTEN, AS160 and IRS1 Expression in the Presence and Absence of Insulin

AS160	**Cont. (+ins)**	**Cont. (-ins)**	**OB (+ins)**	**OB (-ins)**	**AE (+ins)**	**AE (-ins)**	**GT (+ins)**	**GT (-ins)**	**HT (+ins)**	**HT (-ins)**
Rel. Exp.	0.17	1.30	0.445	1.467	0.18	0.19	0.59	2.40	0.10	0.28
	0.14	0.88	0.259	1.201	0.21	0.18	0.68	2.87	0.16	0.31
	0.16	0.87	0.404	1.589	0.21	0.20	0.84	2.65	0.14	0.27
Mean	0.2	1.0	0.369	1.419	0.20	0.19	0.71	2.64	0.13	0.28

SD	0.014	0.246	0.097	0.199	0.015	0.009	0.129	0.236	0.028	0.019
SE	0.008	0.142	0.056	0.115	0.009	0.005	0.075	0.136	0.016	0.011
P-Value	0.025		0.005		0.366		0.004		0.008	
%mRNA Expression	**15**	**100**	**26**	**100**	**105**	**100**	**27**	**100**	**47**	**100**
IRS1 Rel. Exp.	2.08	1.14	0.532	0.995	0.61	0.68	1.65	1.32	0.26	0.43
	2.46	0.87	0.437	0.964	1.35	0.80	1.49	1.46	0.46	0.58
	1.88	1.01	0.503	0.925	1.06	0.94	1.63	1.54	0.31	0.39
Mean	2.1	1.0	0.491	0.961	1.01	0.81	1.59	1.44	0.34	0.46
SD	0.296	0.134	0.049	0.035	0.371	0.126	0.087	0.114	0.103	0.102
SE	0.171	0.077	0.028	0.020	0.214	0.073	0.050	0.066	0.060	0.059
P-Value	0.039		0.004		0.390		0.256		0.224	
%mRNA Expression	**213**	**100**	**51**	**100**	**125**	**100**	**110**	**100**	**74**	**100**

Table 4.6: Assay of Isolated Phenolic Compounds from Abelmoschus esculentus Using Analytical and Preparative HPLC

A.E Phenolic Compound	Ret. Time	Area of STD	Area of Samples	%Assay
Caffeic Acid (F2)	21.4	480.153	3.257	0.678
		480.485	3.074	0.64
Average		480.319	3.166	0.659
Chlorogenic Acid (F1)	18.4	209.1	0.093	0.047
		183.746	0.095	0.048
Average		196.423	0.094	0.048
Benzoic Acid (F3)	21.89	601.798	0.311	0.052
		602.87	0.333	0.055
Average		602.334	0.322	0.054
Ferulic Acid (F4)	24.13	340.06	0.064	0.019
		339.556	0.06	0.018
Average		339.808	0.062	0.018
Vanillin Acid	27.63	187.026	0.302	0.161

(F5)		187.375	0.4	0.214
Average		187.201	0.351	0.188
Quaracetin (F6)	28.8	553.935	0.281	0.051
		554.216	0.279	0.05
Average		554.076	0.28	0.051

Table 4.7: GLUT4 mRNA Expression data for A. esculentus Methanolic Extracts and its Phenolic Compounds

GLUT4 (+insulin)	**CONT.**	**Solvent**	**A. E**	**Caffeic acid**	**Chloro. acid**	**Vanillic acid**	**Ferulic acid**	**Quercetin**	**Benzoic acid**
Sample Rel. Exp	0.84	27.38	0.085	86.31	74.46	121.43	120.73	63.93	51.60
	0.72	25.55	0.073	91.93	56.05	113.85	128.82	66.07	63.69
	0.89	28.14	0.077	80.95	56.17	129.31	124.32	55.61	62.07
Mean	0.8	27.0	0.078	86.40	62.23	121.53	124.63	61.87	59.12
SD	0.000	1.334	0.006	5.488	10.592	7.728	4.054	5.528	6.564
SE	0.000	0.770	0.003	3.169	6.116	4.461	2.341	3.192	3.790
p-value	0.009	0.020	0.323	0.028	0.024	0.183	0.058	0.000	0.349
%Rel.Exp.	**66**	**14**	**114**	**241**	**168**	**140**	**116**	**45**	**70**

GLUT4 (-insulin)	**CONT.**	**Solvent**	**A.E**	**Caffeic acid**	**Chloro. acid**	**Vanillic acid**	**Ferulic acid**	**Quercetin**	**Benzoic acid**
Sample Rel. Exp	1.32	170.62	0.068	21.53	41.85	72.70	98.45	140.04	56.83
	1.14	241.23	0.078	39.68	32.31	113.56	108.26	142.49	130.64
	1.24	171.81	0.059	46.19	37.06	74.27	115.79	133.26	65.66
Mean	1.2	194.6	0.069	35.80	37.07	86.84	107.50	138.59	84.38
SD	0.094	40.428	0.010	12.780	4.768	23.152	8.696	4.783	40.309
SE	0.054	23.341	0.006	7.379	2.753	13.367	5.021	2.761	23.272
%Rel.Exp.	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table 4.8: PTEN mRNA Expression data for A. esculentus Methanolic Extracts and its Phenolic Compounds

PTEN (+insulin)	**CONT.**	**Solvent**	**A.E**	**Caffeic acid**	**Chloro. acid**	**Vanillic acid**	**Ferulic acid**	**Quercetin**	**Benzoic acid**
Sample Rel. Exp	0.22	0.19	0.005	0.22	0.16	0.08	0.00	0.17	0.09
	0.25	0.19	0.004	0.26	0.15	0.07	0.00	0.23	0.12
	0.23	0.18	0.004	0.13	0.15	0.08	0.00	0.20	0.10
Mean	0.2	0.2	0.005	0.20	0.15	0.07	0.00	0.20	0.10
SD	0.018	0.003	0.000	0.066	0.009	0.006	0.000	0.029	0.013
SE	0.011	0.002	0.000	0.038	0.005	0.004	0.000	0.017	0.007
p-value	8.5E-03	4.0E-02	1.0E-04	2.2E-01	8.5E-01	3.3E-02	3.6E-03	1.6E-01	3.9E-03
%Rel.Exp.	**23**	**67**	**2**	**151**	**96**	**28**	**0**	**117**	**21**

PTEN (-insulin)	**CONT.**	**Solvent**	**A.E**	**Caffeic acid**	**Chlorogenic acid**	**Vanillic acid**	**Ferulic acid**	**Quercetin**	**Benzoic acid**
Sample Rel. Exp	1.08	0.24	0.202	0.10	0.13	0.21	0.12	0.13	0.44
	0.88	0.29	0.205	0.16	0.14	0.32	0.14	0.18	0.54
	1.05	0.30	0.209	0.14	0.21	0.27	0.15	0.20	0.48
Mean	1.0	0.3	0.205	0.13	0.16	0.27	0.13	0.17	0.49
SD	0.106	0.030	0.003	0.032	0.043	0.058	0.014	0.035	0.053
SE	0.061	0.017	0.002	0.019	0.025	0.034	0.008	0.020	0.031
%Rel.Exp.	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table 4.9: AS160 mRNA Expression data for A. esculentus Methanolic Extracts and its Phenolic Compounds

AS160 (+insulin)	**CONT.**	**Solvent**	**A.E**	**Caffeic acid**	**Chloro. acid**	**Vanillic acid**	**Ferulic acid**	**Quercetin**	**Benzoic acid**
Sample Rel. Exp	0.91	0.16	0.137	0.13	0.16	0.08	0.11	0.12	0.25
	0.89	0.19	0.113	0.15	0.15	0.08	0.14	0.11	0.27
	0.93	0.25	0.119	0.09	0.16	0.08	0.13	0.11	0.22
Mean	0.9	0.2	0.123	0.12	0.16	0.08	0.12	0.11	0.25
SD	0.021	0.050	0.012	0.034	0.003	0.001	0.016	0.005	0.023
SE	0.012	0.029	0.007	0.020	0.002	0.001	0.009	0.003	0.013
p-value	0.054	0.062	0.074	0.138	0.042	0.034	0.194	0.474	0.021
%Rel.Exp.	**91**	**171**	**126**	**167**	**161**	**30**	**120**	**85**	**138**

AS160 (-insulin)	**CONT.**	**Solvent**	**A.E**	**Caffeic acid**	**Chloro. acid**	**Vanillic acid**	**Ferulic acid**	**Quercetin**	**Benzoic acid**
Sample Rel. Exp	1.03	0.10	0.098	0.06	0.10	0.24	0.09	0.13	0.16
	0.99	0.12	0.090	0.08	0.07	0.34	0.10	0.10	0.21
	0.97	0.13	0.105	0.08	0.12	0.22	0.12	0.17	0.17
Mean	1.0	0.1	0.098	0.07	0.10	0.27	0.10	0.13	0.18
SD	0.030	0.013	0.008	0.009	0.024	0.060	0.013	0.034	0.025
SE	0.018	0.008	0.004	0.005	0.014	0.035	0.008	0.020	0.014
%Rel.Exp.	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table 4.10: IRS1 mRNA Expression data for A. esculentus Methanolic Extracts and its Phenolic Compounds

IRS1 (+insulin)	**CONT.**	**Solvent**	**A.E**	**Caffeic acid**	**Chloro. acid**	**Vanillic acid**	**Ferulic acid**	**Quercetin**	**Benzoic acid**
Sample Rel. Exp	1.79	0.22	0.775	0.75	0.28	0.16	0.47	0.48	0.93
	1.82	0.27	0.621	0.91	0.38	0.24	0.48	0.42	0.89
	1.62	0.20	0.664	0.66	0.37	0.21	0.46	0.35	0.75
Mean	1.7	0.2	0.687	0.77	0.34	0.21	0.47	0.42	0.86
SD	0.108	0.036	0.079	0.127	0.057	0.042	0.014	0.065	0.096
SE	0.062	0.021	0.046	0.073	0.033	0.024	0.008	0.038	0.055
p-value	0.044	0.122	0.104	0.013	0.036	0.070	0.141	0.340	0.034
%Rel.Exp.	**173**	**59**	**150**	**372**	**228**	**56**	**140**	**142**	**247**

IRS1 (-insulin)	**CONT.**	**Solvent**	**A. E**	**Caffeic acid**	**Chloro. acid**	**Vanillic acid**	**Ferulic acid**	**Quercetin**	**Benzoic acid**
Sample Rel. Exp	0.90	0.31	0.389	0.21	0.16	0.23	0.22	0.18	0.23
	0.92	0.56	0.456	0.22	0.13	0.44	0.38	0.46	0.48
	1.20	0.31	0.532	0.19	0.16	0.43	0.40	0.25	0.33
Mean	1.00	0.4	0.459	0.21	0.15	0.37	0.34	0.30	0.35
SD	0.169	0.142	0.072	0.013	0.015	0.118	0.099	0.145	0.129
SE	0.097	0.082	0.041	0.008	0.009	0.068	0.057	0.084	0.074
%Rel.Exp.	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

تأثير مستخلصات نبات الباميه، العرن مثلث الأوراق،الريحان والعكوب على فعالية جينات البروتينات التي تؤثر على فعالية إشارة الانسولين ونقل السكر في خلايا العضلات

سالي عفيف سليمان الجوده

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دكتور اياد العلي

ملخص

داء السكري هو اضطراب استقلابي يتميز بارتفاع مستويات الجلوكوز في الدم، ويُعد من الأمراض المزمنة الشائعة التي تؤثر على ملايين الأشخاص حول العالم. يشتق اسم "داء السكري" من الكلمة اليونانية "ديابيتس" التي تعني "المرور عبر" أو "النفاذ"، وكلمة "مليتوس" اللاتينية التي تعني "حلو". يُصنف داء السكري إلى نوعين رئيسيين: النوع الأول والنوع الثاني .السكري من النوع الأول يحدث عندما يهاجم جهاز المناعة خلايا بيتا في البنكرياس، المسؤولة عن إنتاج الأنسولين، مما يؤدي إلى نقص حاد في الأنسولين. يظهر هذا النوع عادةً في مرحلة الطفولة أو المراهقة. أما السكري من النوع الثاني، الذي يُعد الأكثر شيوعًا، فهو ناتج عن مقاومة الأنسولين، حيث يصبح الجسم غير قادر على استخدام الأنسولين بشكل فعال، ما يؤدي إلى تراكم السكر في الدم. يرتبط هذا النوع بشكل رئيسي بسوء التغذية ونمط الحياة الخامل .في هذا السياق، تلعب البروتينات مثلGLUT4 دورًا رئيسيًا في تنظيم مستويات الجلوكوز في الدم وهو ناقل الجلوكوز الرئيسي الذي يتواجد في الأنسجة الحساسة للأنسولين مثل العضلات والعظام. عند تحفيز الخلايا بواسطة الأنسولين أو التمرين البدني، ينتقل ناقل الجلوكوز الرئيسي إلى سطح الخلايا لامتصاص الجلوكوز من الدم. وبالتالي، فإن أي خلل في وظيفته يمكن أن يؤدي إلى اضطراب في تنظيم مستويات الجلوكوز، وهو ما يساهم بشكل كبير في تطور السكري من النوع الثاني. أظهرت الأبحاث الحديثة أهمية دراسة المسارات الجزيئية التي تؤثر في حركة GLUT4 في خلايا العضلات مثل خلايا (L6) من أجل فهم آلية تأثير الأدوية أو العلاجات الطبيعية على التحكم في مستوى الجلوكوز وتحديد كيفية تأثير هذه العلاجات على المسارات الجزيئية مثل مسار Myc الذي يرتبط بتنظيم ناقل الجلوكوز الرئيسي يمكن أن يساعد في تطوير علاجات أكثر فعالية لمقاومة الأنسولين.

في السنوات الأخيرة، بدأت الاهتمامات تتزايد بشأن استخدام النباتات الطبية في علاج داء السكري، وذلك بسبب المخاوف المتعلقة بالآثار الجانبية طويلة الأمد للأدوية الكيميائية. على الرغم من أن الأدوية الكيميائية مثل السلفونيل يوريا والثيازوليدين ديونات تُستخدم في علاج السكري، إلا أن استخدامها المستمر قد يسبب آثارًا جانبية ضارة مثل السرطان أو تلف الكبد. نباتات مثل البامية Abelmoschus esculentus والعكوب Gundelia tournefortii والعرن مثلث الاوراق triquetrifolium Hypericum أظهرت فعاليتها في علاج داء السكري. تحتوي هذه النباتات على مركبات بيولوجية نشطة مثل الفلافونويدات والتانينات، التي تلعب دورًا مهمًا في تعزيز وظيفة البنكرياس وتنظيم امتصاص الجلوكوز في الأمعاء. بالإضافة إلى ذلك، فهي تمتاز بخصائص مضادة للأكسدة، مضادة للالتهابات، وتدعم صحة القلب والكبد.تُظهر الدراسات ايضا أن البامية تحتوي على مركبات تساعد في تقليل مستويات الجلوكوز في الدم، مما يجعلها خيارًا واعدًا لإدارة داء السكري من النوع الثاني. كما أظهرت دراسة أن مستخلصات العكوب يمكن أن تحسن امتصاص الجلوكوز عن طريق زيادة حركة GLUT4 إلى غشاء الخلية، مما يساعد في تنظيم مستويات السكر في الدم. أما العرن مثلث الاوراق، فقد أظهرت أبحاث متعددة أنه يحتوي على مركبات مضادة للأكسدة ومضادة للالتهابات، مما يعزز من فعاليته في علاج السكري. نبات Ocimum basilicum ، أو الريحان المقدس. يُستخدم الريحان في الطب التقليدي لعلاج العديد من الأمراض، بما في ذلك السكري. يحتوي الريحان على مركبات نشطة مثل الفلافونويدات، التربين، والأحماض الفينولية التي تساهم في تحسين استجابة الأنسولين وتنظيم مستويات الجلوكوز في الدم. الدراسات الحديثة تشير إلى أن الريحان المقدس قد يلعب دورًا في تحفيز حركة ناقل الجلوكوز الرئيسي إلى غشاء الخلية، مما يعزز امتصاص الجلوكوز ويحسن حساسية الأنسولين. يُعتبر ذلك تطورًا مهمًا، حيث يساعد في التخفيف من تأثير مقاومة الأنسولين التي تعتبر من الأسباب الرئيسية لتطور السكري من النوع الثاني.

وفي مناطق مثل فلسطين، حيث يعتمد العديد من الأشخاص على العلاجات الطبيعية بسبب محدودية الوصول إلى الرعاية الصحية الحديثة، تلعب النباتات الطبية دورًا كبيرًا في إدارة الأمراض المزمنة مثل السكري. تحتوي فلسطين على أكثر من 700 نوع من النباتات الطبية المستخدمة في علاج العديد من الأمراض، بما في ذلك السكري.بالتالي، تزداد أهمية البحث في النباتات الطبية كبديل للأدوية التقليدية، إذ أنها تقدم حلولًا طبيعية وآمنة مع آثار جانبية أقل، مما يساهم في تحسين جودة حياة مرضى السكري في جميع أنحاء العالم.

النتائج

دَرَسَتِ الدراسة السمية الخلوية والإمكانات العلاجية لاستخلاصات Abelmoschus esculentus، Hypericum triquetrifolium، Ocimum basilicum و Gundelia tournefortii في خلايا العضلات L6 GLUT4 أظهر اختبار 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide أن جميع المستخلصات غير سامة حتى تركيز 1000 ميكروغرام/مل، مما يضمن حيوية الخلايا لإجراء تجارب الفعالية عند نفس التركيز. أظهر تحليل كروماتوغرافيا السوائل عالية الأداء لمستخلص البامية غنيه بالمركبات الفينولية، حيث كان حمض الكافيك هو الأكثر وفرة (0.66%)، يليه حمض الفانيليك (0.19%)، مع وجود كميات معتدلة من حمض الكلوروجينيك، وحمض الفيروليك، وحمض البنزويك، وحمض الكيرسيتين. تُعرف هذه الفينولات بخصائصها المضادة للأكسدة والالتهابات والسكري، مما يبرز النشاط البيولوجي المحتمل لهذا المستخلص. كما تُبرز الدراسة إمكانات مستخلصات النباتات ومركباتها الفينولية في تنظيم المؤشرات الرئيسية لعملية استقلاب الجلوكوز، بما في ذلك ناقل الجلوكوز الرئيسي ونظير الفوسفاتاز والتنسين و ركيزةAkt بوزن 160 كيلو دالتون و ركيزة مستقبل الأنسولين 1، في خلايا عضلات L6. حيث أن المستخلصات الميثانولية لأربعة أنواع نباتية (الريحان، البامية، العكوب، العرن مثلث الاوراق) تؤثر على تعبير الجينات الرئيسية في مسار الإشارة للأنسولين في خلايا L6 العضلية. أظهرت النتائج أن العرن مثلث الاوراق يعزز بشكل كبير من تعبير ناقل الجلوكوز الرئيسي عند تحفيز الأنسولين، مما يشير إلى دوره المحتمل في تحسين امتصاص الجلوكوز، بينما قلل العكوب من تعبير ناقل الجلوكوز الرئيسي ، مما قد يشير إلى تأثير مثبط. كما أدى كل من العكوب، الريحان، والبامية إلى تقليل تعبير نظير الفوسفاتاز والتنسين عند وجود الأنسولين، مما يعزز من إشارات الأنسولين، في حين أن العرن مثلث الاوراق كان له تأثير معتدل. أما بالنسبة لـ Akt بوزن 160 كيلو دالتون ، فقد أظهر البامية تأثيرًا قويًا في استعادة تعبيره، بينما أظهرت العكوب و العرن مثلث الاوراق تأثيرات أقل قوة. بالنسبة لـ ركيزة مستقبل الأنسولين1، كان البامية الأكثر قدرة على استعادة تعبيره، في حين أن الريحان والعرن مثلث الاوراق قللا من مستوياته، مما قد يشير إلى تأثيرهما المثبط على إشارات الأنسولين. علاوة على ذلك، تبين أن المركبات الفينولية في البامية، مثل حمضي الكافيك والكلوروجينيك، تلعب دورًا رئيسيًا في تعزيز تعبير ناقل الجلوكوز الرئيسي ، في حين أن الكيرسيتين كان له تأثير مثبط قوي. تشير هذه النتائج إلى أن بعض المستخلصات النباتية، خاصة البامية و العرن مثلث الاوراق، قد تكون مرشحة لعلاجات طبيعية لتحسين حساسية الأنسولين وتنظيم استقلاب الجلوكوز، لكن هناك حاجة إلى مزيد من الأبحاث لتوضيح آليات تأثيرها.

الخلاصة

هدفت الدراسة إلى فحص تأثيرات أربع نباتات على إشارات الأنسولين في خلايا L6 GLUT4 تم استخدام الاستخلاصات من نباتات البامية ،العرن مثلث الأوراق، الريحان، والعكوب وتحليل التعبير الجيني للبروتينات المرتبطة بإشارات الأنسولين مثل ونظير الفوسفاتاز والتنسين ، ركيزة Akt بوزن 160 كيلو دالتون، ركيزة مستقبل الأنسولين 1 و ناقل الجلوكوز الرئيسي أظهرت النتائج تأثيرات مختلفة لهذه النباتات على التعبير الجيني لتلك البروتينات، مع تأثيرات ملحوظة على تعزيز أو تثبيط إشارات الأنسولين. بالإضافة إلى ذلك، تم تحديد المركبات الفعالة من البامية باستخدام أظهر تحليل كروماتوغرافيا السوائل عالية الأداء ، حيث كانت تأثيرات النباتات على التعبير الجيني مرتبطة بتفاعلات مختلفة مع إشارات الأنسولين. أظهرت النتائج أن بعض النباتات يمكن أن تعزز إشارات الأنسولين، بينما قد تثبطها أخرى. تشير الدراسة إلى إمكانيات استخدام الاستخلاصات النباتية في تحسين أو تنظيم التفاعلات الأيضية، ما يعزز من فهمنا للأثر المحتمل للنباتات في العلاج الطبيعي للاضطرابات الأيضية.

الكلمات المفتاحية: داء السكري من النوع الثاني، إشارات الأنسولين، نواقل الجلوكوز، النباتات الطبية، المركبات الكيميائية النباتية (الفيتوكيميكال).