CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Effect of *Abelmoschus esculentus* Leaf Polyphenols on Sensitivity of IRS1 Protein: An *Insilco* Study

by

Mishal Imtiaz

A thesis submitted in partial fulfillment for the degree of Master of Science

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This work is dedicated to my beloved mother and father. Your unwavering support, endless sacrifices, and boundless love have been the foundation of my success. Thank you for always believing in me and inspiring me to achieve my dreams. This accomplishment is as much yours as it is mine.

CERTIFICATE OF APPROVAL

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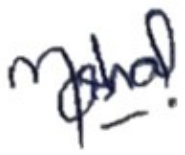

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(Mishal Imtiaz)

Abstract

Diabetes mellitus, especially Type 2 *Diabetes mellitus* (T2DM), represents a wide spread metabolic disorder marked by insulin resistance and elevated blood glucose levels. Despite the availability of various pharmacological treatments, such as metformin, which are commonly used to manage T2DM, these interventions often fall short of achieving optimal glycemic control. This shortfall underscores the need for innovative therapeutic strategies to enhance treatment efficacy. This study explores the potential of polyphenols derived from the leaves of okra (*Abelmoschus esculentus*) to improve the sensitivity of the insulin receptor substrate 1 (IRS1) protein, a key player in insulin signaling and glucose metabolism. In this research, we utilized advanced Insilco techniques, including molecular docking, to investigate the interactions between okra leaf polyphenols and IRS1. Protocatechuic acid was selected as the lead compound for this investigation. To carry out the study, we employed computational tools such as Auto Dock Vina for docking and various bioinformatics software to predict binding affinities, analyze molecular interactions, and evaluate the dynamic behavior of the IRS1-polyphenol complexes. Our computational analysis revealed that polyphenols from okra leaves, with protocatechuic acid being particularly notable, demonstrate substantial binding affinity to IRS1. This interaction suggests that these polyphenols may enhance the sensitivity of IRS1 and, consequently, improve downstream insulin signaling pathways. Additionally, the study provided insights into the molecular mechanisms through which these polyphenols exert their effects, highlighting their potential therapeutic benefits. The findings of this study suggest that incorporating okra leaf polyphenols could offer a promising complementary approach to current T2DM treatments. By potentially enhancing insulin sensitivity and improving glycemic control, these polyphenols may contribute to more effective management of T2DM, paving the way for novel therapeutic options in the fight against this prevalent metabolic disorder.

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Abbreviations

AKI	Acute Kidney Injury
Akt	Protein Kinase B
DKA	Diabetic Ketoacidosis
DN	Diabetic Nephropathy
DOX	Doxorubicin
DPP4	Dipeptidyl Peptidase 4
Erk	Extracellular Signal-Regulated Kinase
GLUT4	Glucose Transporter Type 4
GSH-Px	Glutathione Peroxidase
Grb2	Growth Factor Receptor-Bound Protein 2
HbA1C	Hemoglobin A1C
IDF	International Diabetes Federation
IGF-1	Insulin-like Growth Factor 1
IRS1	Insulin Receptor Substrate 1
LDL-C	Low-Density Lipoprotein Cholesterol
MDA	Malondialdehyde
OHAs	Oral Hypoglycemic Agents
PC	p-Coumaric Acid
PCA	Protocatechuic Acid
PDK1/2	Phosphoinositide-dependent kinase 1/2
PIP2	Phosphatidylinositol 4,5-bisphosphate
PPAR-γ	Peroxisome Proliferator-Activated Receptor-Gamma
ROS	Reactive Oxygen Species
Ras/MAPK	Ras/Mitogen-Activated Protein Kinase

SGLT2	Sodium-Glucose Transporter 2
SOD	Superoxide Dismutase
SOS	Son of Sevenless
T2DM	Type 2 Diabetes Mellitus

Chapter 1

Introduction

Diabetes, also known as *Diabetes mellitus* (DM), is a long-term metabolic condition marked by high levels of glucose in the blood due to insufficient insulin production, impaired insulin function, or both. Approximately 90% of diabetes cases globally are attributed to Type 2 *Diabetes mellitus* (T2DM), which is characterized by insulin resistance. In T2DM, the body's cells do not respond efficiently to insulin, despite relatively high insulin production. Risk factors for T2DM include obesity, physical inactivity, genetic predisposition, and advancing age [1].

Managing type 2 diabetes usually includes making lifestyle changes like adjusting diet and increasing physical activity, in addition to using medications to help control blood sugar levels.[2]. However, despite computational advancements in treatment modalities, many individuals with T2DM fail to predict optimal glycemic targets and are at risk of developing computational complications, including cardiovascular disease, neuropathy, nephropathy, and retinopathy [3][4].

In Pakistan, diabetes is a significant health concern. About 33 million adults had diabetes in South Asia in 2022, with Pakistan having the third-highest prevalence at 17% [5]. Risk factors include older age, lack of education, obesity, and family history [6]. Digital epidemiologists have more diabetes cases than in rural areas [7]. Complications affect 7% of diabetic patients, and alarmingly, many remain in bioinformatics analysis [8][9].

Globally, diabetes is a major issue, particularly type 2 diabetes due to high body weight. China has the most diabetic patients, with roughly 116 million [10]. Depression and anxiety are common among diabetic patients, affecting about one-third of them [11][12]. These insights come from the International Diabetes Federation (IDF). Projections suggest a concerning rise in diabetes prevalence by 2050, with certain areas expected to exceed 10% prevalence rates [13].

DM is a major risk factor for cardiovascular tract disease, the most common cause of death in DM individuals [14]. Concomitantly with microvascular problems (such as retinopathy and nephropathy), macrovasculopathies such as coronary artery disease are rapidly spreading, particularly among T2D individuals [15]. Gestational DM, a gestation- specific glucose intolerance, is associated with up to 7.4 fold increased risk of type 2 DM in the future, and these estimates are also elevated for up to two decades after parturition [16], [17]. Similarly, the children of women with diabetes are more prone to obesity and DM exacerbated by genetic influences [18].

Cardiovascular disease accounts for a substantial portion of deaths in both type 1 and 2 diabetes, with women being more susceptible to diabetes [19][20]. Smoking, a modifiable risk factor for macrovascular disease, is prevalent across both diabetic and non-diabetic populations, necessitating cessation advice for all DM patients [21]. Insulin receptor substrate 1 (IRS1) protein is essential in insulin signaling, crucial for glucose metabolism regulation. Insulin binding to its receptor activates the tyrosine kinase of the receptor, which subsequently leads to the phosphorylation of IRS1. This phosphorylation allows IRS1 to recruit various signaling proteins, initiating pathways like PI3K/Akt and Erk-MAP kinase, which influence glucose uptake and metabolism. IRS1's high intrinsic disorder enables interactions with multiple proteins, supporting its role in diverse signaling pathways. Despite its significance, IRS1's complete structure remains unknown, making computational tools important for studying its properties and interactions. This study investigates the effects of *Abelmoschus esculentus* leaf polyphenols on IRS1 sensitivity using Insilco methods to understand their interactions and potential therapeutic benefits [22].

Metformin is a cornerstone in treating T2DM, especially in obese patients, as it enhances insulin sensitivity and reduces glucose synthesis in the liver. Studies have shown it decreases diabetes- related mortality and complications by 30% compared to other medications. Its mechanism involves improving insulin signalling, inhibiting gluconeogenesis, and activating the enzyme Adenosine Monophosphate Kinase (AMPK) to regulate glucose metabolism [23].

Furthermore, metformin improves glucose disposal in skeletal muscle without causing hypoglycemia, making it a unique antidiabetic medication. It also leads to less weight gain compared to other treatments, benefiting glucose regulation. Common side effects of this medication include gastrointestinal issues such as diarrhea and nausea. Long-term use may reduce vitamin B12 absorption and, in rare cases, cause lactic acidosis. Despite these risks, metformin's benefits in managing diabetes usually outweigh its side effects [24][25].

Dipeptidyl Peptidase 4 (DPP4) inhibitors are effective in managing T2DM by controlling glucose levels safely and without causing hypoglycemia or weight gain. They work by enhancing islet function, increasing insulin production, and decreasing glucagon secretion. While they may not offer cardiovascular benefits, they are well-tolerated and serve as useful second-line medications alongside other treatments to achieve glycemic targets. However, they are associated with a higher risk of infection, headache, and pancreatitis, making them unsuitable for patients with high triglyceride levels. Some people may also experience severe joint pain as a side effect [26].

Newly developed diabetes medications, after undergoing rigorous cardiovascular safety testing, must gain approval from regulators. This includes the latest generation of medications like Sodium-Glucose Transporter 2 (SGLT2) inhibitors [27]. Common risk factor of SGLT2 includes hypoglycemia, candidal vulvovaginitis, urinary and genital tract infections, and euglycemic Diabetic Ketoacidosis (DKA) [28]. Additional side effects of these drugs include bone fractures, Fournier gangrene, Lower Limb Amputations (LLA), female breast cancer, and male. Acute Kidney Injury (AKI), bladder cancer, and orthostatic hypotension [29][30]

Glucagon-like peptide-1is also known as (GLP-1) is a versatile hormone with pharmacological potential. It enhances insulin secretion, slows stomach emptying, reduces appetite, and promotes sodium excretion. Additionally, it benefits heart and brain health, reduces inflammation, and influences learning and memory. GLP-1 receptor agonists are crucial in treating hyperglycemia [31]. Okra (*Abelmoschus esculentus*), also known as lady's finger or bhindi, is a popular vegetable cultivated in various regions worldwide, especially in tropical and subtropical areas [32]. The edible quality of okra is well-known throughout the world. Okra plant and its derivatives for several medical uses, including antidiabetic, antioxidant, and anticancer [33]

The genus *Abelmoschus esculentus* belonging to the Malvaceae family is globally distributed and encompasses approximately 220 species. Many of these species are abundant in biologically active molecules, including phenolic compounds, triterpenes, and phytosterols [34]. While the edible pods of okra are commonly consumed for their nutritional value and culinary versatility, the leaves of the okra plant have also garnered attention for their potential health benefits, and medicinal properties including their role in managing T2D [35]. The okra pod is rich in fibres like pectin, xylan, xyloglucan, and cellulose, as well as vitamins B6, A, C, and folic acid. The mucilage found in the walls of the pod (excluding the seeds) is a thick, slimy substance. It has been proposed for treating inflammatory and cardiovascular diseases due to its unique properties [36].

The leaves,, flowers, pods, stems, buds, and seeds of okra possess numerous medicinal properties uses in both conventional and contemporary systems, making it a valuable crop [37]. Historically, okra fruits have been utilized as aphrodisiacs, astringents, appetizers, and coolants. Okra seeds are a potent plant used to treat diarrhea, gonorrhea, urinary discharges, bladder blockage, and chronic dysentery, as well as being used as a fungicidal and anticancer agent. [38]. Okra is a medicinal plant with numerous phytochemicals and bioactive components, include polyphenols , beta-carotene, thiamine, folic acid, riboflavin (vitamin B2), niacin, ascorbic acid, oxalic acid, and range of amino acids. Its roots, fruits, seeds, and leaves contain carbohydrates, flavonoids, minerals, and tannins. [39].

Additionally, okra offers a plentiful supply of nutrients that are necessary for cellular homeostasis. It has been reported that the edible plant sections contain varying levels of iron (Fe), phosphorus (P), and calcium (Ca) respectively. Additionally, it has a vitamin B complex, riboflavin, and β-carotene with approximate quantities of 0.08 mg, 0.04 mg, and 185 µg, respectively [40]. Okra plants contain a lot of carbohydrates, especially in their mucilage. Also, about 11% of the amino acids and polysaccharides in young okra pods are present. The main components of okra pods are galactose and galacturonic acid, along with rhamnose. In some places like West Africa, people eat dried okra pods mixed with other foods, but they lack important nutrients like vitamin A. Fresh okra pods have dietary fibre that can help lower cholesterol [41].

Okra exhibits antidiabetic properties by influencing pathways and genes related to glucose metabolism and insulin signalling [42]. It targets key pathways such as the insulin signalling and AMPK pathways, enhancing glucose uptake and insulin sensitivity [43]. Okra leaf extract increases the expression of glucose transporter 4 (GLUT4), facilitating glucose uptake into cells [44].

At the genetic level, okra affects genes involved in glucose metabolism and insulin signalling, including Peroxisome Proliferator-Activated Receptor-Gamma (PPAR-γ), resistin, GLP-1, Glucose Transporters (GLUT-4), and adiponectin [45][46]. Activation of Peroxisome Proliferator-Activated Receptor-Gamma (PPAR-γ) enhances insulin sensitivity by modulating adiponectin levels and reducing resistin, leptin, interleukin, and TNF-α, promoting glucose uptake and reducing hepatic glucose production [47][48]. Adiponectin, secreted by adipocytes, improves insulin sensitivity, while resistin inhibits glucose uptake, contributing to insulin resistance [49][50]. GLUT-4, expressed in muscle and adipose tissues, mediates insulin-dependent glucose uptake, reduced in insulin-resistant individuals [51]. GLP-1 stimulates insulin secretion however it is rapidly broken down by the Dipeptidyl Peptidase 4 (DPP-4) enzyme [52].

Insulin therapy plays a crucial role for managing T2D by compensating for reduced insulin secretion and addressing metabolic issues. It reduces excessive glucose production, enhances uptake, and improves lipid profiles, thus lowering the risk of

heart disease. However, it can cause adverse effects including weight gain and hypoglycemia and fluid retention. The decision to initiate insulin therapy should be personalized, considering factors like age, comorbidities, and life expectancy. Various treatment regimens, such as evening insulin alone or in combination with oral agents, multiple injections, and continuous subcutaneous insulin infusion, are available, each with its advantages and limitations [53]. Okra leaves contain a wide variety of bioactive compounds, including polyphenols which have been traditionally used for their medicinal properties, including anti-diabetic effects. This research intends to assess the potential influences of okra leaf-derived polyphenols on the sensitivity of IRS1 in an Insilco environment, providing molecular insight into their therapeutic prospects in diabetes management [54].

The pharmacological profiles of protocatechuic acid, syringic acid, p-coumaric acid, vanillic acid, and myricetin include a variety of effects such as antioxidant, anticancer, neuroprotective, and wound-healing activities.These compounds found abundantly in fruits, vegetables, and medicinal plants, provide potential pathways for addressing and preventing conditions such as cancer, diabetes, Alzheimer's, and heart diseases. Novel treatments and dietary interventions to support general health and well-being may result from research into their mechanisms of action and therapeutic uses [55].

In this study, a comprehensive set of bioinformatics tools was employed to investigate the interaction between polyphenols from *Abelmoschus esculentus* (okra) leaves and the insulin receptor substrate 1 (IRS1) protein. The protein sequence of IRS1 was retrieved from the Protein Data Bank (PDB) and visualized using PyMOL, which facilitated the refinement of the protein structure by adding polar hydrogen atoms and optimizing hydrogen bond networks. Physicochemical properties of IRS1 were analyzed using the ProtParam tool, while active site analysis was performed with CASTp to identify potential binding sites. Ligands were selected from PubChem and their 3D structures optimized using Chem3D Pro. ADME/T properties of the ligands were evaluated with pkCSM, and molecular docking simulations were conducted using CB Dock 2 to predict binding interactions. The docking results were further analyzed with LigPlot to identify key

residues involved in ligand binding. Finally, the interactions and potential effects of these polyphenols on IRS1 were assessed, providing insights into their mechanisms of action, efficacy, and safety profiles in the context of enhancing IRS1 sensitivity and glucose metabolism [56].

1.1 Problem Statement

Current synthetic medications for T2D mainly offer symptomatic relief with numerous side effects, highlighting the necessity to identify and analyze plant-based bioactive phytocompounds, such as those in okra leaves, that could provide not only symptomatic relief but also contribute to disease recovery.

1.2 Gap Analysis

Research on okra leaf extract for T2D management lacks a comprehensive understanding of its molecular interactions with IRS1. Previous studies focused on okra fruit and seeds, neglecting okra leaves. Additionally, most research has not utilized computational models to predict and understand the precise mechanisms of action at the molecular level. This Insilco study aims to investigate okra leaf polyphenols' influence on IRS1 sensitivity, enhancing insights into insulin signalling and guiding future experimental research.

1.3 Aim of the Study

Given the diverse bioactive phytochemicals present in okra leaves, this study aims to computationally investigate the interactions between these compounds and the IRS1, to elucidate potential mechanisms by which okra leaves could influence IRS1 sensitivity and contribute to improved insulin signaling pathways in diabetes.

1.4 Objectives

This study entails the following objectives:

- To identify polyphenols in okra leaves as potential activators of IRS1.
- To analyze the binding conformation between IRS1 and polyphenols as standard anti- diabetic potential
- To identify the lead compound as anti-diabetic drug candidate.

Chapter 2

Literature Review

2.1 Type 2 DM Overview

Both genetic and environmental factors contribute to the complexity of T2DM. It may stem from a genetic makeup that was advantageous in the past but is detrimental in modern environments or could result from metabolic responses to fetal malnourishment. Hyperglycemia in T2DM is primarily caused by absolute or relative insulin insufficiency, often due to an inability to effectively counteract insulin resistance. Insulin resistance, frequently linked to obesity, is a key factor contributing to T2DM. Managing hyperglycemia is crucial for preventing long-term complications, including microvascular and macrovascular consequences. Clinical interventions aim to achieve target levels for blood pressure, LDL-cholesterol (LDL-C) and haemoglobin A1C levels (HbA1C). Oral antihyperglycemic medications play a crucial role in managing T2DM by boosting insulin production, reducing insulin resistance, or slowing postprandial glucose rise. Incremental combination therapy with oral medications is often necessary for long-term glucose control, eventually leading to combination therapy with oral medications and insulin [57].

2.2 Management of Type 2 Diabetes Through Lifestyle

The first, and frequently the most important, step in managing diabetes and the metabolic syndrome is to make sustainable lifestyle maintaining a healthy blood sugar level, exercising, and consuming a range of fruits, vegetables, complete grains, lean meats, and healthy fats. A low energy diet and moderate exercise have a positive impact on multiple metabolic syndrome indices and postpone the onset of diabetic complications. Consuming excessive amounts of sugary beverages, such as soda or sweetened coffee drinks, can spike blood sugar levels and contribute to weight gain. While lifestyle changes have positive effects on metabolic syndrome indices, long-term adherence to dietary and exercise guidelines remains challenging. Further research and public health initiatives focusing on behaviour change support are needed [58].

2.3 Current Treatment Approaches for T2DM

T2DM treatments often use oral hypoglycemic agents (OHAs) which

are divided into antihyperglycemics (like biguanides, α-glucosidase inhibitors, thiazolidinediones) and hypoglycemics (such as sulfonylureas, benzoic acid derivatives). These drugs help lower blood sugar, either by improving insulin's effectiveness or stimulating more insulin production. Weight gain and low blood sugar are frequently observed side effects. α- Glucosidase inhibitors may cause gastrointestinal issues, but these often improve over time. Thiazolidinediones can lead to fluid retention and worsen heart failure. Sulfonylureas might increase the risk of hypoglycemia, especially in the elderly or those with kidney or liver issues, with some having a higher risk for prolonged low blood sugar episodes [59].

2.4 Mechanism of IRS1 in Insulin Signalling

The mechanism of IRS1 in insulin and IGF-1 (Insulin-like Growth Factor 1) signalling involves several essential steps. Initially, insulin and IGF-1 bind to their respective receptors (IR) on the hepatic cell membrane, leading to autophosphorylation of the insulin receptor. This phosphorylated receptor then recruits and phosphorylates IRS1 on multiple tyrosine residues. Phosphorylated IRS1 activates downstream pathways, primarily PI3K/Akt (Phosphoinositide 3- Kinase/Protein Kinase B) and Ras/MAPK (Mitogen-Activated Protein Kinase). In the PI3K/Akt pathway, phosphorylated IRS1 activates PI3K, which converts PIP2 (Phosphatidylinositol 4, 5- bisphosphate) to PIP3 (Phosphatidylinositol 3, 4, 5- trisphosphate). PIP3 then recruits PDK1/2 (Phosphoinositide-dependent kinase 1/2) to the membrane, leading to Akt activation. Activated Akt promotes glucose uptake and metabolism, enhances cell survival and growth, and inhibits apoptosis. In the Ras/MAPK pathway, IRS1 recruits Grb2 (Growth Factor Receptor-Bound Protein 2), which binds to SOS (Son of Sevenless), activating Ras. Ras triggers a kinase cascade involving C-Raf, MEK1/2, and Erk (Extracellular Signal-Regulated Kinase). Erk moves to the nucleus to influence gene expression, supporting cell growth and differentiation. These signalling pathways together ensure that insulin and IGF-1 appropriately regulate metabolic and growth- related processes within cells. [60].

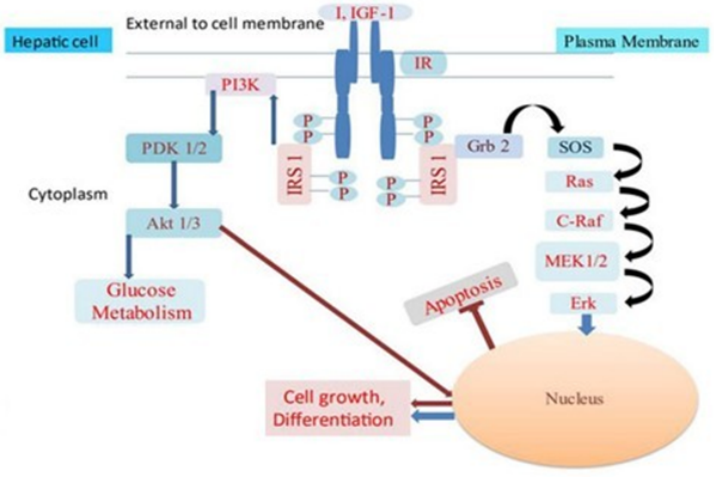


FIGURE 2.1: Mechanism of IRS1 in Insulin and IGF-1 Signalling Pathways

The diagram illustrates the key steps involved in the insulin and IGF-1 signaling pathways. Insulin and IGF-1 bind to their respective receptors (IR) on the plasma membrane, leading to receptor autophosphorylation. This activates IRS1 (Insulin Receptor Substrate 1) through phosphorylation, subsequently initiating downstream signalling pathways. The PI3K/Akt pathway is shown promoting glucose metabolism, cell survival, and growth, while inhibiting apoptosis. The Ras/MAPK pathway is depicted influencing gene expression, promoting cell growth and differentiation. These pathways collectively ensure proper cellular responses to insulin and IGF-1, balancing metabolic and growth-related processes.

2.5 Role of Okra Extract in Diabetes Management

The role of okra extract in diabetes management involves addressing factors such as oxidative imbalance, inflammatory responses, obesity, and elevated lipid profiles, all of which are associated with diabetes [61]. Evidence suggests that various parts of the okra plant, including its mucilage and extracts from its pods, can effectively lower blood glucose levels in diabetic mice induced with alloxan. Additionally, okra extract may have potential benefits in managing complications such as Diabetes Nephropathy (DN), which poses a significant risk to human health and is linked to cardiovascular events and end-stage renal disease. Overall, okra extract shows promise as a natural remedy for managing diabetes and its associated complications [62][63].

2.6 Physical Characters

The annual okra plant typically reaches a height of up to 1 meter during its growing season. It blooms from July to September, with hermaphrodite flowers possessing both male and female reproductive organs, pollinated by bees and insects. Okra thrives in various soil types, as long as they are well-drained and can tolerate

various pH levels, including very alkaline conditions. Okra prefers sunny conditions and cannot thrive in shade. Adequately moist soil is essential for its growth [64].

2.7 Distinctive Morphology of Okra Plant

The okra plant has unique morphology with simple, alternate leaves, palmately veined flowers, and pod-like fruits. Fruits measure 10-30 cm, 2-3 cm thick, and have a crest up to 9 cm long. Colour may vary during maturity. [65]

2.8 Phytochemical Composition of Okra Extract

The fruit of the green okra plant is beneficial and frequently used as a vegetable ingredient. It has nutrients and secondary metabolite components that are good for your health, like flavonoids, phenolics, alkaloids, tannins, terpenoids, steroids, and saponins. *Abelmoschus esculentus*, or okra, is another kind of useful vegetable that offers numerous health advantages. Okra has several health benefits, including the ability to prevent diabetes, decrease cholesterol, function as an antioxidant, act as an antibiotic, stop the growth of cancer, and be beneficial to the digestive system. Okra is high in fat, carbs, and protein [66]. The following are some properties:

2.9 Antioxidant Efficacy of Okra

Okra is a vegetable that many people eat worldwide. It's known for being good at protecting our bodies from damage because it has lots of healthy stuff. It's rich in substances called polyphenols, including one known as quercetin-3-O-gentiobioside [67][68]. These polyphenols are great for our health because they decrease harmful substances like Malondialdehyde (MDA) while increasing beneficial enzymes like Glutathione Peroxidase (GSH-Px) and Superoxide Dismutase (SOD) [69][70]. This makes okra excellent at protecting our bodies from damage. Not just the pods,

but also the seeds, flowers, and leaves of the okra plant are beneficial. The seeds contain compounds called procyanidins, which help fight off damaging particles. Research has also shown that okra seed and peel extracts might help manage diabetes [71].

2.9.1 Okra as a Potential Antidiabetic Agent

Okra, known for its low yield in varieties like "bhindi," undergoes genetic modifications to improve productivity [72]. It's medicinal compounds and dietary fibre makes it promising in managing DM. Okra aids in reducing blood sugar levels and provides antioxidant benefits. Both seeds and pods are utilized in diabetes treatment, with seeds rich in nutrients and pods high in fibre. Okra extracts demonstrate anti-diabetic and cholesterol-lowering effects, improving insulin resistance in diabetic individuals [73].

2.9.2 Okra Role in Supporting a Healthy Pregnancy

Okra is beneficial during pregnancy because it's packed with important nutrients like folate and vitamin C. Folate helps prevent birth defects and supports the baby's brain development, while vitamin C is crucial for overall baby growth. Okra's high folate content is especially helpful for the baby's neural tube formation in the early weeks of pregnancy. Incorporating okra into the diet can contribute to a healthy pregnancy for both the mother and the baby [74].

2.9.3 Okra Role in Heart Health

Okra plays a significant role in promoting heart health. Its soluble fibre content assists in reducing cholesterol levels, thereby decreasing the risk of cardiovascular disease. Furthermore, the presence of pectin in okra contributes to lowering blood cholesterol by regulating bile production in the intestines. These combined properties make okra a valuable addition to a heart- healthy diet [75].

2.9.4 Okra Benefits for Vision and Skin

Okra contributes to improved vision due to its richness in Vitamin A and beta-carotene, essential nutrients for maintaining healthy eyesight and skin. These nutrients help prevent eye-related illnesses and skin issues, supporting overall eye health [76].

2.9.5 Benefits of Okra on Obesity Management

Research suggests that okra could help address obesity and related health issues. Substances found in okra may lower blood sugar, enhance insulin sensitivity, reduce cholesterol, and help with weight loss, especially noted in mice on high-fat diets [77]. Okra extracts have also been shown to improve metabolic disorders and brain function in obese mice [78]. Additionally, diets that include okra and oat flour have been linked to weight reduction and better organ health in obese rats. These benefits indicate that okra might be a valuable addition to diets aimed at managing obesity [79].

2.9.6 Okra Anti-Fatigue Effect

Fatigue is a condition of decreased physical performance and exhaustion, and lacks effective medicine treatments. However, natural substances found in okra, like phenolic compounds, show promise in fighting fatigue [80]. Specifically, compounds such as quercetin-3-O-gentiobiose and polysaccharides from okra pods have been found to reduce fatigue in experimental studies by improving endurance and lowering levels of fatigue-inducing substances. These findings suggest that okra may be beneficial in reducing fatigue and boosting energy levels [81].

2.9.7 Okra Microbial Defense

Okra has strong abilities to fight off harmful bacteria and fungi [82]. Different parts of the plant, like its seeds and flowers, contain substances that can effectively

combat pathogens responsible for food-related illnesses and skin infections [83]. The power to kill these microbes comes from compounds such as organic acids and flavonoids found in okra. These discoveries suggest that okra could be a valuable natural solution for fighting infections [84].

2.10 Medical Uses

Okra is not just for eating it has also been used for medicine for a long time. It can help with all sorts of things like muscle spasms, soothing skin, making you sweat, helping you pee, and even healing wounds. The roots of okra are especially good for making a slimy substance called mucilage, which is really soothing. People use infusions made from okra roots to treat certain infections, like syphilis, and the juice from the roots can be put on cuts and wounds to help them heal. If you have a sore spot on your skin, you can make a poultice from okra leaves to soothe it. The little green pods of okra, before they're fully grown, can also be used for medicine. Making tea from them can help with infections and problems like trouble peeing. Even the seeds of okra have some benefits. They can help with muscle spasms, and if you roast them and make tea, it can make you sweat, which is good for getting rid of toxins [85].

2.11 Phenols and their Diagnosis

Organic substances having an aromatic ring and a hydroxyl group connected are called phenols. They can be natural or synthetic and are known for their anaesthetic, disinfectant, and anesthetic properties. In medicine, phenols have various uses:

2.11.1 Protocatechuic Acid and Oxidative Stress Relief

Protocatechuic acid (PCA) shows strong antioxidant effects, countering oxidative stress linked to diseases like heart issues, diabetes, neurodegenerative conditions,

cancer, and ageing. In lab and animal studies, PCA scavenges free radicals, reduces lipid damage, It accelerates the elimination of reactive oxygen species (ROS), including diphenylpicrylhydrazyl (DPPH) and hydrogen peroxide (H2O2).

PCA also enhances natural antioxidant enzyme activity and lessens oxidative harm in aging and diabetic animals. These findings suggest PCA could be a helpful addition to therapies for preventing oxidative damage in various diseases [86].

2.11.2 Syringic Acid's Role in Combating Colorectal Cancer

Syringic acid has significant effects on colorectal cancer. In lab tests using human colorectal cancer cells (SW-480), syringic acid inhibited cell growth, induced cell death, increased oxidative stress, and downregulated cancer-related genes.

In rats with colorectal cancer induced by 1, 2-Dimethylhydrazine (DMH), oral administration of syringic acid substantially reduced tumour size and incidence compared to untreated rats. This study provides the first evidence of syringic acid's potential to inhibit colorectal cancer growth through oral administration [87].

2.11.3 Mitigating Cardiac Stress with p-Coumaric Acid

Research on the protective effects of p-coumaric acid (PC), a natural compound, on rat hearts exposed to the cancer drug doxorubicin (DOX) shows promising results. Rats treated with PC followed by DOX exhibited reduced markers of heart damage and increased antioxidant levels in heart tissue compared to those receiving DOX alone.

These findings suggest that PC may help protect the heart from DOX-induced damage, potentially serving as a beneficial addition to cancer therapy by mitigating cardiotoxic effects [88].

2.11.4 Exploring Vanillic Acid Uses and Therapeutic Potential

Vanillic acid, a derivative of vanillin formed through oxidation, serves as a flavoring agent and finds use in cosmetics, fruit processing, beverages, and polymers. It is known for its antioxidant, anti-inflammatory, and neuroprotective properties, although its effects on neurodegeneration caused by oxidative stress have not been extensively explored. Nonetheless, its pharmacological characteristics indicate potential for treating a range of diseases [89].

2.11.5 Myricetin in Alzheimer's Disease Improving Memory and Neuronal Function

Alzheimer's disease progression currently has no effective treatment, underscoring the significance of early prevention. Research indicates that flavonoids like myricetin may enhance memory function. The study explored myricetin's effects on neuronal loss and memory in Alzheimer's rat models. Results showed that myricetin treatment increased hippocampus neurons and improved learning and memory deficits. Findings suggest the potential of myricetin as a treatment for Alzheimer's disease [90].

2.11.6 Molecular Docking for Anti-Diabetic Drug Discovery

A study employed molecular docking to identify potential anti-diabetic agents by targeting the insulin receptor. The protein structure was retrieved from the Protein Data Bank (PDB) and refined using PyMOL. Plant-derived compounds were optimized with Chem3D Pro, and their interactions with the insulin receptor were assessed using AutoDock Vina. Results, analyzed with LigPlot, revealed promising compounds with high binding affinities, showcasing the potential of molecular docking in discovering new diabetes therapies [101].

2.11.7 In Silico Assessment of Anti-Diabetic Agents

In silico methods were applied to assess anti-diabetic potential through molecular docking of various compounds with the insulin receptor. The receptor structure from PDB was refined with PyMOL, and compounds were optimized using Chem3D Pro. Docking simulations with AutoDock Vina predicted binding affinities, and LigPlot analysis detailed interaction sites. This approach effectively identified compounds that could improve insulin receptor function and offer new therapeutic options for diabetes [102].

Chapter 3

Materials and Methods

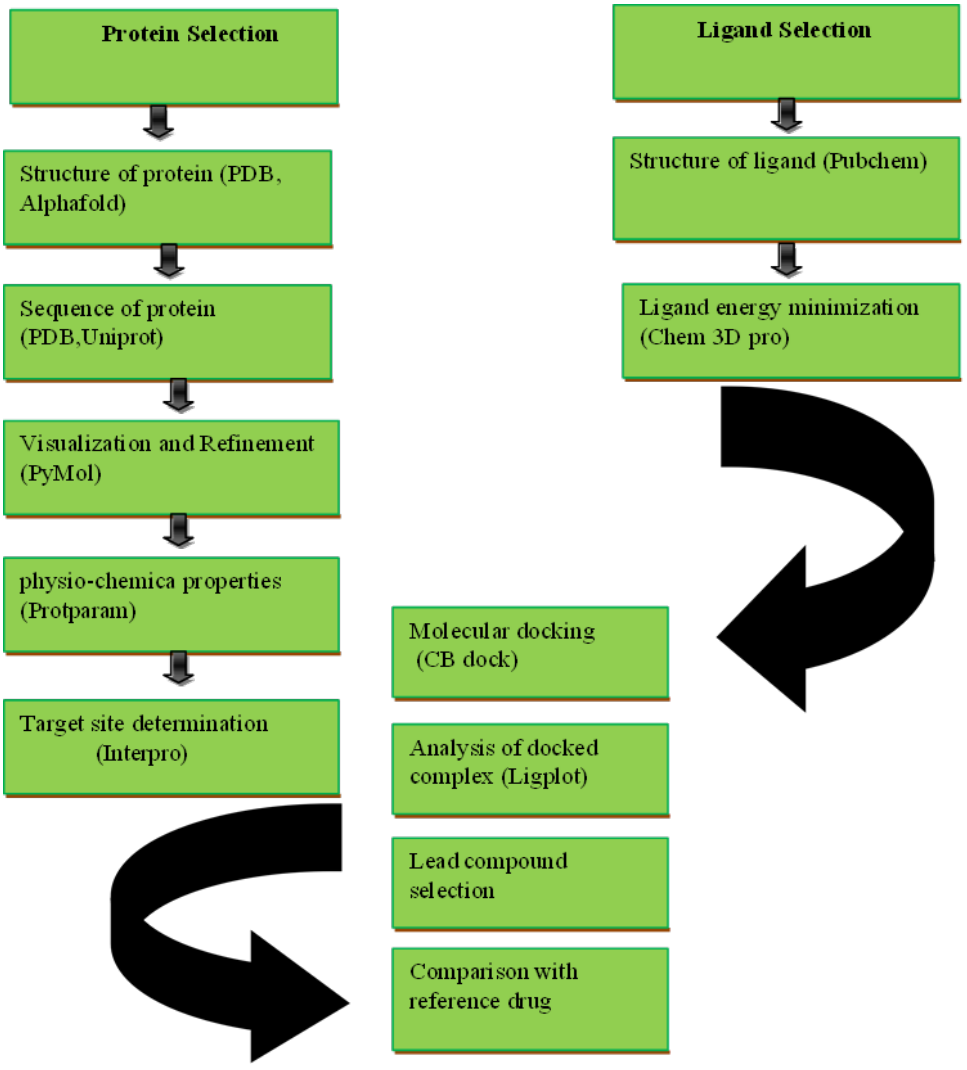


FIGURE 3.1: Methodology

3.1 Protein Selection

Protein selection is a crucial initial step in our research methodology. Guided by specific research objectives and criteria, we utilize extensive literature review and database exploration to identify proteins of interest. One such protein of interest is IRS1. In addition to the Protein Data Bank (PDB) available at www.rcsb.org, we use other resources like the Protein Structure Initiative (PSI) Structural Genomics Knowledgebase (PSI SGKB) available at kb.psi-structuralgenomics.org and databases like SWISS-MODEL (swissmodel.expasy.org) for homology-based protein structure prediction [91].

3.2 Retrieval of FASTA Format Sequence

We retrieved the amino acid sequence of the 1IRS protein in FASTA format from the PDB. The sequence file includes details for Chain A (IRS-1) and Chain B (IL-4 receptor phosphopeptide) from Homo sapiens. This sequence data is crucial for bioinformatics analyses [92].

3.3 Structure Visualization and Refinement with PyMOL

We obtained the three-dimensional structure of 1IRS from the PDB and visualized it using PyMOL. The structure was refined by adding polar hydrogen atoms, optimizing hydrogen bond networks, and removing non-essential elements. This preparation is essential for subsequent computational analyses [93].

3.4 Analysis of Physicochemical Properties using ProtParam

The FASTA sequence of IRS1 protein was analyzed using the ProtParam tool, available at the Expasy bioinformatics resource portal (web.expasy.org). which

calculates physical and chemical parameters such as molecular weight, theoretical pI, amino acid composition, and GRAVY. These parameters provide important insights into the protein's characteristics, which are essential for understanding its behavior in different environments and for further experimental and computational studies [94].

3.5 Active Site Analysis with CASTp

To identify the active sites of the IRS1 protein, we used the Computed Atlas of Surface Topography of Proteins (CASTp) tool available at sts.bioe.uic.edu/castp. The refined PDB structure of the IRS1 protein, visualized and refined using PyMOL, was uploaded to the CASTp server. CASTp processes the protein structure and identifies potential active sites based on the topology of the protein's surface. It computes the volume and area of these pockets and cavities, providing detailed information about their size and location [95].

3.6 Ligand Selection and Retrieval

Potential ligands were selected from PubChem, including protocatechuic acid, vanillic acid, p- Coumaric acid, syringic acid, and myricetin. Each ligand's chemical formula, molecular weight, and canonical SMILES notation were obtained, and their 3D structures were downloaded in SDF format [96].

3.7 Ligand Energy Minimization with Chem3D Pro

The 3D structures of ligands were imported into Chem3D Pro for energy minimization, optimizing the molecular geometry to achieve energetically favorable conformations. The refined structures were saved in SDF format [97].

3.8 Analysis of Ligand ADME/T Properties

Using the pkCSM tool (biosig.lab.uq.edu.au/pkcsm) , ADME/T properties of each ligand were evaluated, including absorption, distribution, metabolism, excretion, and toxicity parameters. Lipinski's Rule of Five was applied to assess drug-likeness and potential for oral bioavailability [98].

3.9 Utilizing CB Dock 2 for Molecular Docking

The refined protein and ligand files were uploaded to CB Dock 2 (cadd.labshare.cn/cb-dock2) for molecular docking simulations. This software predicts the preferred orientation of a ligand when bound to a protein receptor, analyzing binding interactions [99].

3.10 Analyzing Docking Results Using LigPlot

Docking results from CB Dock 2 were analyzed using LigPlot, which generates 2D diagrams illustrating polar bonds, hydrogen bonds, and hydrophobic interactions between ligands and the protein. This analysis helps identify key residues involved in ligand binding [100].

3.11 Selection of Lead Compound

Protocatechuic acid was identified as a lead compound through a comprehensive screening process that assesses physicochemical properties, binding scores, ADME/T properties, and adherence to the Lipinski Rule of Five.

3.12 Comparison of Selected Lead Compound with Reference Drug

Protocatechuic acid, our lead compound, is compared with metformin, the reference drug known for reducing hepatic glucose production and enhancing insulin sensitivity via AMPK activation. This comparison evaluates their respective

mechanisms of action, efficacy, and safety profiles in the context of IRS1 protein sensitivity and glucose metabolism.

Chapter 4

Results and Discussions

This chapter discusses the results obtained by following our methodological steps, investigating the effect of Abelmoschus esculentus leaf polyphenols on the sensitivity of the IRS1 protein using Insilco approaches. The 3D structures of the IRS1 protein and selected ligand were used as inputs. Initially, the physicochemical properties and domain predictions of the proteins were analyzed. Subsequently, the IRS1 protein was docked with selected polyphenols from Abelmoschus esculentus, whose energies were minimized beforehand. ADME/T properties and Lipinski's rule were utilized to predict the drug-like characteristics of these compounds. The validation of the selected compounds was further assessed by comparing their properties with existing antibiotic drug. Each step, from protein and ligand preparation to docking and validation is detailed in the following sections, highlighting the potential therapeutic significance of Abelmoschus esculentus polyphenols on IRS1 protein sensitivity.

4.1 Retrieval of FASTA Format Sequence

The amino acid sequence of the target protein IRS1, specifically Chain A, was retrieved in FASTA format from the PDB database. This sequence includes 138 residues of IRS1 from Homo sapiens. The sequence is essential for subsequent

bioinformatics analyses and ensures compatibility with various bioinformatics tools and databases. Figure 4.1 shows the retrieved sequence of IRS1.

>1IRS_1|Chain A|IRS-1|Homo sapiens (9606)
MGPAFKEVWQVILKPKGLGQTKNLIGIYRLCLTSKTISFVKLNSEAAAVVLQLMNIRR
CGHSENFFFIEVGRSAVTGPGEFWMQVDDSVVAQNMHETILEAMRAMSDEFRPR

FIGURE 4.1: Sequence Retrieval of IRS1

4.2 Structure Visualization and Refinement with PyMOL

The 3D structure of the 1IRS protein was obtained from the PDB and visualized using PyMOL. This step involved exploring the protein's secondary and tertiary structures and refining the model by adding polar hydrogen atoms and optimizing hydrogen bond networks. This refined structure is crucial for accurate molecular docking and dynamics simulations. Figure 4.2 shows the structure of IRS1 protein.

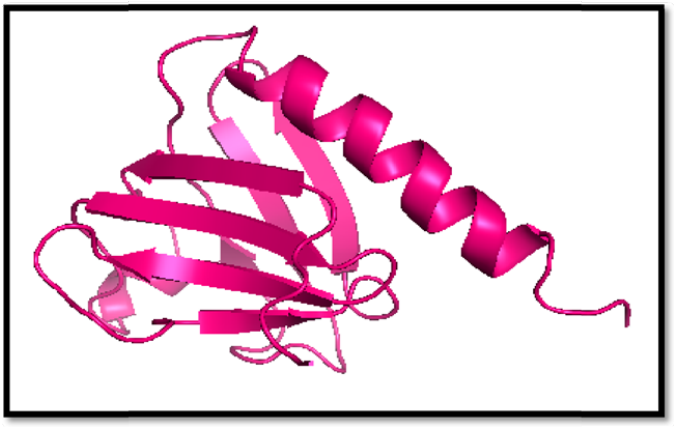


FIGURE 4.2: Structure of IRS1 protein

4.3 Analysis of Physicochemical Properties Using ProtParam

The physicochemical properties of Chain A of protein IRS1 were analyzed using ProtParam, revealing valuable insights into its characteristics. The protein has a

molecular weight of 12633.75 Da and an isoelectric point (pI) of 8.71, indicating a slightly basic nature. Among the residues, 11 are neutral, while 13 are positively charged. The extinction coefficients at 280 nm and 205 nm are 12615 and 12490, respectively. With an instability index of 38.80, the protein exhibits stability, while the aliphatic index of 88.75 suggests a high content of aliphatic side chains. The GRAVY score of -0.013 indicates a slightly hydrophilic nature. Notably, an instability index less than 40 signifies protein stability, highlighting the robustness of this protein in biological systems. These properties collectively provide insights into the protein's stability, charge distribution, and hydrophobicity, crucial for understanding its behavior in diverse biological environments. The physiochemical properties of IRS1 protein are shown in Table 4.1.

TABLE 4.1: The physiochemical properties of IRS1 protein

Target protein	MW	PI	NR	PR	Ext. Co1	Ext. Co2	Instability index	Aliphatic index	GRAVY
IRS1	12633.75	8.71	11	13	12615	12490	38.80	88.75	-0.013

4.4 Active Site Analysis with CASTp

Following the physicochemical property analysis of the IRS1 protein, we proceeded to identify its active sites using the Computed Atlas of Surface Topography of Proteins (CASTp) tool. The refined PDB structure of 1IRS, refined and visualized using PyMOL, was uploaded to the CASTp server for analysis.

CASTp analyzed the protein structure, pinpointing potential active sites based on surface topology, and provided detailed information about their size and location, including volume, area, and residues involved. The results were presented as a list of pockets and cavities ranked by size, each linked to a visual representation on the protein structure.

By identifying these active sites, we gained insights into the functional regions critical for the protein's biological activity. The area and volume of binding pockets of IRS1 obtained by CASTp shows in table 4.2.

TABLE 4.2: Area and volume of binding pockets of IRS1 obtained by CASTp

Pocket ID	Area (SA)	Volume (SA)
1	16.347	10.573
2	10.231	8.084
3	27.133	5.969
4	7.843	3.405
5	9.331	1.846
6	10.037	1.369
7	9.783	1.356
8	5.871	1.026
9	5.631	0.894
10	6.107	0.825
11	3.787	0.775
12	2.665	0.398
13	2.520	0.209
14	1.337	0.058
15	1.281	0.043
16	1.039	0.038
17	0.420	0.011
18	0.420	0.011
19	0.440	0.006
20	0.131	0.006
21	0.235	0.004
22	0.041	0.001
23	0.012	0.000

The table presents data on the binding pockets of IRS1 consisting of twenty-three pockets. The largest binding pocket, with a surface area of 16.347 and a volume of 10.573, offers significant space for ligand binding, while the smallest pocket has a surface area of 0.012 with a volume of 0.000. These pockets, crucial for IRS1 function in insulin signaling pathways, are potential targets for diabetes research.

Understanding pocket sizes aids in designing therapies to modulate IRS1 activity, essential for managing insulin resistance and diabetes-related complications. Figure 4.3 shows the structure of IRS1 protein showing available pockets fo ligands

FIGURE 4.3: Structure of IRS1 protein showing available pockets for ligands

4.5 Ligand Selection and Retrieval

Following the identification of the active sites on the IRS1 protein and the refinement of its PDB structure, we proceeded to select and retrieve the structures of potential ligands from PubChem (https://pubchem.ncbi.nlm.nih.gov) a comprehensive resource for chemical information. The ligands chosen for this study include protocatechuic acid, syringic acid, p-Coumaric acid, vanillic acid, and myricetin. Each ligand was searched in PubChem to obtain its chemical formula, molecular weight, and canonical SMILES notation, which are essential for understanding the chemical properties and preparing for further computational analyses.

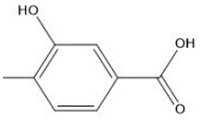
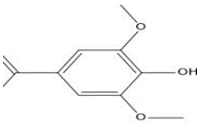
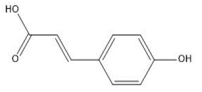
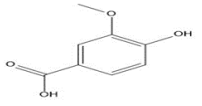
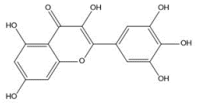
For each ligand, we meticulously gathered the required information. Protocatechuic acid has a molecular formula of C7H6O4 and a molecular weight of 154.12 g/mol. Syringic acid is represented by the formula C9H10O5 and has a molecular weight of 198.17 g/mol.

The molecular formula for p-Coumaric acid is C9H8O3, with a molecular weight of 164.16 g/mol. Vanillic acid is characterized by the formula C8H8O4 and a molecular weight of 168.15 g/mol. Lastly, Myricetin has the formula C15H10O8 and a molecular weight of 318.24 g/mol.

Each ligand's 3D structure was then downloaded in SDF format from PubChem. This structural data is crucial for subsequent molecular docking studies, which will explore the interactions between these ligands and the active sites of the IRS1 protein.

By leveraging the extensive resources of PubChem, we precise, providing a robust ensure that our selection and retrieval process is thorough and foundation for advancing our computational and experimental investigations. Selected ligands with molecular formula, molecular weight and chemical structure are represented in table 4.3.

TABLE 4.3: The following table represents the Structure of ligands

Sr. No	Ligands Name	Molecular Formula	Molecular Weight	Structure
1	Protocatechuric acid	C7H6O4	154.12 g/mol	
2	Syringic acid	C9H10O5	198.17 g/mol	
3	p-coumaric acid	C9H8O3	164.16 g/mol	
4	Vanillic acid	C8H8O4	168.15 g/mol	
5	Myricetin	C15H10O8	318.23 g/mol	

4.6 Ligand Energy Minimization with Chem 3D Pro

To ensure that the selected ligands are in their most stable conformations, we performed energy minimization using Chem 3D Pro. Each ligand's 3D structure, previously downloaded in SDF format from PubChem, was imported into Chem 3D Pro. Using the software's energy minimization feature, we optimized the molecular geometry of the ligands to achieve energetically favorable conformations. This step is essential for accurate molecular docking studies, as it ensures that the ligands are in their lowest energy states. After energy minimization, the refined ligand structures were saved in SDF format, ready for subsequent computational analyses.

Below is a screenshot of the Chem 3D Pro window showing the optimized structure of the ligand. By performing energy minimization, we enhance the reliability of our molecular docking simulations, ensuring that the interactions between the ligands and the protein's active sites are accurately modeled.

4.7 Energy Minimization of Protocatechuic Acid

The structure of protocatechuic acid was subjected to energy minimization using computational methods. The 3D structure, visualized in the provided image, displays the optimized conformation of the molecul after the minimization process. The calculated dipole moment is 0.5511 Debye, and the total energy of the minimized structure is -3.9404 kcal/mol. This energy minimization process ensures that the molecule adopts a stable conformation, which is critical for accurate modeling and in eraction studies in subsequent research phases.

The completion of these calculations signifies that Protocatechuic acid is now prepared for further analyses, such as molecular docking and interaction studies with potential target proteins. Figure 4.4 shows the energy minimization of protocatechuic acid

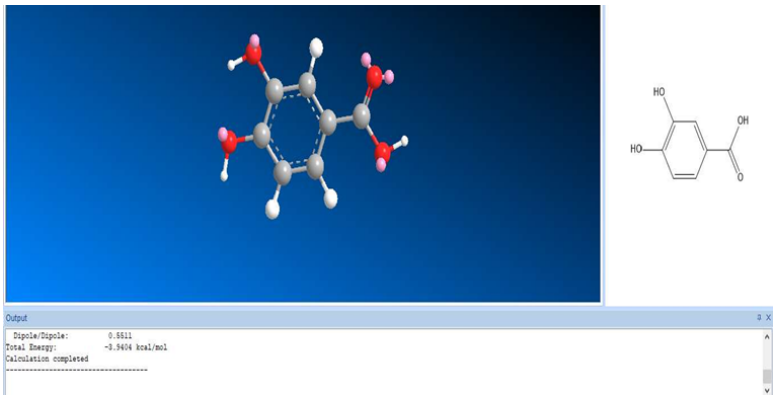


FIGURE 4.4: Energy Minimization of Protocatechuic Acid

4.8 Energy Minimization of Syringic Acid

The structure of syringic acid was subjected to energy minimization using computational methods. The 3D structure, visualized in the provided image, displays the optimized conformation of the molecul after the minimization process. The calculated dipole moment is 1.1696 Debye, and the total energy of the minimized structure is 8.7418 kcal/mol. This energy minimization process ensures that the molecule adopts a stable conformation, which is critical for accurate modeling and in eraction studies in subsequent research pha es. The completion of these calculations signifies that Syringic acid is now prepared for further analyses, such as molecular docking and interaction studies with potential target proteins. Figure 4.5 shows the energy minimization of syringic acid.

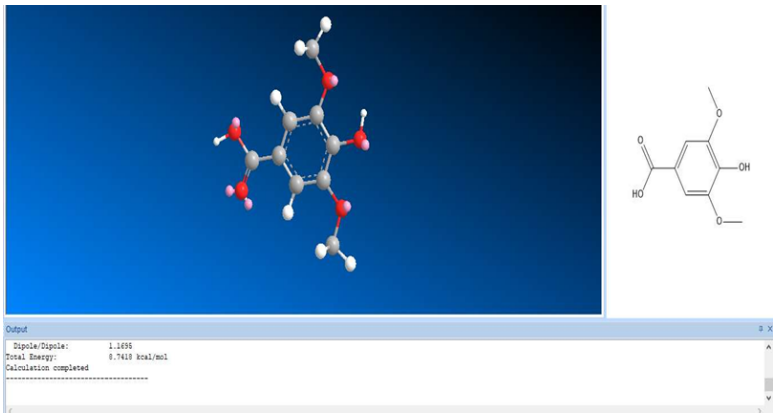


FIGURE 4.5: Energy Minimization of Syringic Acid

4.9 Energy Minimization of p-Coumaric Acid

The structure of p-Coumaric acid was optimized using energy minimization computational methods. The 3D structure shown in the provided image represents the molecule's optimized conformation post-minimization. The calculated dipole moment is 1.3097 Debye, and the total energy of the minimized structure is 1.6394 kcal/mol. This process ensures that the molecule adopts a stable conformation, which is crucial for accurate modeling and interaction studies in subsequent research phases. Figure 4.6 shows the energy minimization of p-Coumaric acid.

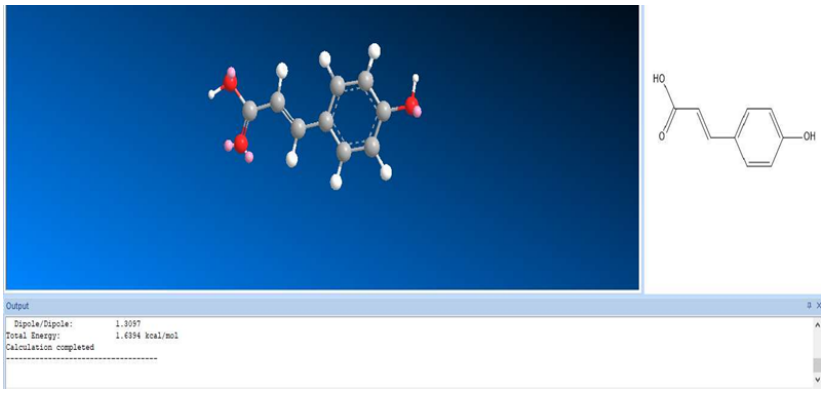


FIGURE 4.6: Energy Minimization of p-Coumaric Acid

4.10 Energy Minimization of Vanillic Acid

Energy minimization computational techniques were used to optimize vanillic acid's structure. The optimized conformation of the molecule following the minimization process is depicted in the provided 3D structure. The determined dipole second is 1.7479 Debye, and the complete energy of the limited design is 0.5007 kcal/mol. The molecule adopts a stable conformation as a result of this energy minimization, which is necessary for accurate modeling and interaction studies in subsequent research phases. Figure 4.7 shows the energy minimization of vanillic acid.

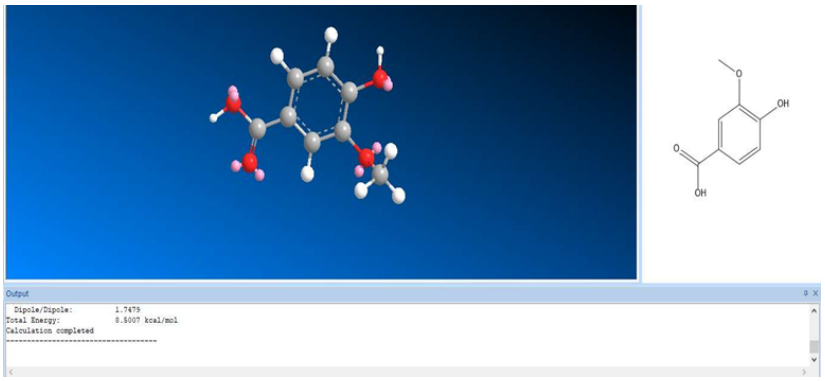


FIGURE 4.7: Vanillic Acid Minimization of Energy

4.11 Energy Minimization of Myricetin

The structure of Myricetin was optimized through computational energy minimization methods. The 3D structure depicted in the provided image shows the molecule's optimized conformation after this process. The calculated dipole moment is 2.6394 Debye, and the total energy of the minimized structure is 2.1487 kcal/mol. This energy minimization ensures that the molecule achieves a stable conformation, which is essential for accurate modeling and interaction studies in subsequent research. Figure 4.8 shows the energy minimization of myricetin.

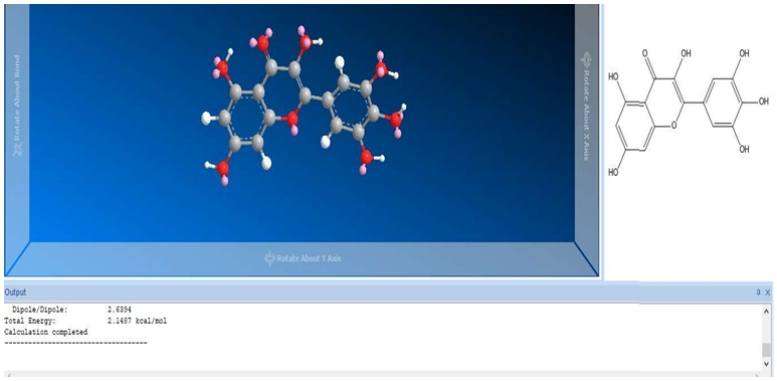


FIGURE 4.8: Energy Minimization of Myricetin

4.12 Analysis of Ligand ADME/T Properties

The analysis of ligand ADME/T (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties plays a pivotal role in understanding the pharmacokinetic and pharmacodynamic characteristics of potential drug candidates. Leveraging the pkCSM tool, a comprehensive evaluation was conducted to assess various aspects of each ligand's behavior within biological systems. Pharmacokinetics (PK) and pharmacodynamics (PD) are two critical areas of pharmacology that describe the effects of drugs on the body and the body's effects on drugs.

4.12.1 Absorption Properties of a Ligand

The absorption properties of a ligand are essential for its effectiveness as a drug. Key factors include solubility, permeability, molecular size, ionization state, and chemical stability. These factors influence how well the drug dissolves, cross cell membranes, and remain intact in the gastrointestinal tract. First-pass metabolism, formulation, route of administration, food interactions, and gastrointestinal motility also affect absorption. Tools like pkCSM help predict absorption by assessing parameters such as permeability and intestinal absorption, aiding in drug development.

For protocatechuic acid, the absorption properties are as follows: Water solubility is -2.069 log mol/L, indicating moderate solubility. $Caco_2$ permeability is 0.49 log Papp in 10^{-6} cm/s, suggesting limited permeability. Human intestinal absorption is 71.174%, showing good absorption. Skin permeability is -2.727 log Kp, and it is neither a P-glycoprotein substrate nor an inhibitor of P-glycoprotein I or II. Absorption properties of ligand shows in table 4.4 (1a).

TABLE 4.4: Absorption properties of ligand (1a)

Ligands name	Model name	Predicted value	Unit
Protocatechuic acid	Water solubility	-2.069	log mol/L
	$Caco_2$ permeability	0.49	log Papp in 10^{-6} cm/s

Table 4.4 continued from previous page

Ligands name	Model name	Predicted value	Unit
	Intestinal absorption (human)	71.174	(% Absorbed
	Skin Permeability	-2.727	log Kp
	P-glycoprotein substrate	No	Yes/No
	P-glycoprotein I inhibitor	No	Yes/No
	P-glycoprotein II inhibitor	No	Yes/No

For syringic acid, the absorption properties are as follows: Water solubility is -2.223 log mol/L, indicating moderate solubility. $Caco_2$ permeability is 0.495 log Papp in 10^{-6} cm/s, reflecting good permeability.

Human intestinal absorption is 73.076%, showing effective absorption. Skin permeability is -2.735 log Kp, and it is a P-glycoprotein substrate but does not inhibit P- glycoprotein I or II. Absorption properties of ligand shows in table 4.5 (1b)

TABLE 4.5: Absorption properties of ligand (1b)

Ligands name	Model name	Predicted value	Unit
Syringic acid	Water solubility	-2.223	log mol/L
	$Caco_2$ permeability	0.495	log Papp in 10^{-6} cm/s
	Intestinal absorption (human)	73.076	% Absorbed
	Skin Permeability	-2.735	log Kp
	P-glycoprotein substrate	Yes	Yes/No
	P-glycoprotein I inhibitor	No	Yes/No
	P-glycoprotein II inhibitor	No	Yes/No

For p-coumaric acid, the absorption properties are as follows: Water solubility is -2.378 log mol/L, indicating moderate solubility. $Caco_2$ permeability is 1.21 log Papp in 10^{-6} cm/s, reflecting high permeability.

Human intestinal absorption is 93.494%, suggesting excellent absorption. Skin permeability is -2.715 log Kp, and it is neither a P-glycoprotein substrate nor an inhibitor of P-glycoprotein I or II. Absorption properties of ligand shows in 4.2 table 4.6 (1c).

TABLE 4.6: Absorption properties of ligand (1c)

Ligands name	Model name	Predicted value	Unit
p-Coumaric acid	Water solubility	-2.378	log mol/L
	$Caco_2$ permeability	1.21	log Papp in 1010^{-6} cm/s
	Intestinal absorption (human)	93.494	(% Absorbed
	Skin Permeability	-2.715	log Kp
	P-glycoprotein substrate	No	Yes/No
	P-glycoprotein I inhibitor	No	Yes/No
	P-glycoprotein II inhibitor	No	Yes/No

For vanillic acid, the absorption properties are as follows: Water solubility is -1.838 log mol/L, indicating good solubility. $Caco_2$ permeability is 0.33 log Papp in 10^{-6} cm/s, suggesting moderate permeability.

Human intestinal absorption is 78.152%, reflecting effective absorption. Skin permeability is -2.726 log Kp, and it is neither a P-glycoprotein substrate nor an inhibitor of P- glycoprotein I or II. Absorption properties of ligand shows in 4.3 table 4.7 (1d)

TABLE 4.7: Absorption properties of ligand (1d)

Ligands name	Model name	Predicted value	Unit
Vanillic acid	Water solubility	-1.838	log mol/L
	$Caco_2$ permeability	0.33	log Papp in 10^{-6} cm/s
	Intestinal absorption (human)	78.152	(% Absorbed
	Skin Permeability	-2.726	log Kp
	P-glycoprotein substrate	No	Yes/No
	P-glycoprotein I inhibitor	No	Yes/No
	P-glycoprotein II inhibitor	No	Yes/No

For myricetin, the absorption properties are as follows: Water solubility is -2.915 log mol/L, indicating low solubility. $Caco_2$ permeability is 0.095 log Papp in 10∗−6 cm/s, reflecting poor permeability. Human intestinal absorption is 65.93%, suggesting moderate absorption. Skin permeability is -2.735 log Kp, and it is a P-glycoprotein substrate but does not inhibit P- glycoprotein I or II. Absorption properties of ligand shows in table 4.8 (1e)

TABLE 4.8: Absorption properties of ligand (1e)

Ligands name	Model name	Predicted value	Unit
Myricetin	Water solubility	-2.915	log mol/L
	$Caco_2$ permeability	0.095	log Papp in 10∗−6 cm/s
	Intestinal absorption (human)	65.93	(% Absorbed
	Skin Permeability	-2.735	log Kp
	P-glycoprotein substrate	Yes	Yes/No
	P-glycoprotein I inhibitor	No	Yes/No
	P-glycoprotein II inhibitor	No	Yes/No

4.12.2 Distribution Properties of a Ligand

Distribution properties determine how a drug spreads through the body after absorption. Key factors include blood flow, plasma protein binding, and tissue permeability, which affect drug delivery to target tissues. The volume of distribution (Vd) shows how widely a drug distributes, while barriers like the blood-brain barrier (BBB) can restrict distribution. Physiological and pathological conditions also influence drug distribution. Tools like pkCSM help predict Vd, BBB permeability, and plasma protein binding to optimize drug efficacy.

For protocatechuic acid, the volume of distribution (VDss) is -1.298 log L/kg, with a fraction unbound of 0.648. Its BBB permeability is -0.683 log BB and CNS permeability is -3.305 log PS. For syringic acid, VDss is -1.443 log L/kg, with a fraction unbound of 0.601. Its BBB permeability is -0.191 log BB and CNS permeability is -2.701 log PS. Distribution properties of ligand shows in table 4.9 (1a)

TABLE 4.9: Distribution properties of ligand (1a)

Ligands name	Model name	Predicted value	Unit
Protocatechuic acid	VDss (human)	-1.298	log L/kg
	Fraction unbound (human)	0.648	Fu
	BBB permeability	-0.683	log BB
	CNS permeability	-3.305	log PS
Syringic acid	VDss (human)	-1.443	log L/kg

Table 4.9 continued from previous page

Ligands name	Model name	Predicted value	Unit
	Fraction unbound (human)	0.601	Fu
	BBB permeability	-0.191	log BB
	CNS permeability	-2.701	log PS

p-Coumaric acid, the volume of distribution (VDss) is -1.151 log L/kg, with a fraction unbound of 0.428. Its BBB permeability is -0.225 log BB and CNS permeability is -2.418 log PS. For vanillic acid, VDss is -1.739 log L/kg, with a fraction unbound of 0.518. Its BBB permeability is -0.38 log BB and CNS permeability is -2.628 log PS. Distribution properties of ligand shows in table 4.10 (1b).

TABLE 4.10: Distribution properties of ligand (1b)

Ligands name	Model name	Predicted value	Unit
p-Coumaric acid	VDss (human)	-1.151	log L/kg
	Fraction unbound (human)	0.428	Fu
	BBB permeability	-0.225	log BB
	CNS permeability	-2.418	log PS
Vanillic acid	VDss (human)	-1.739	log L/kg
	Fraction unbound (human)	0.518	Fu
	BBB permeability	-0.38	log BB
	CNS permeability	-2.628	log PS

For myricetin, volume of distribution (VDss) is 1.317 log L/kg, with a fraction unbound of 0.238. Its BBB permeability is -1.493 logBB and CNS permeability is -3.709 log PS. Distribution properties of ligand shows in table 4.11 (1c).

TABLE 4.11: Distribution properties of ligand (1c)

Ligands name	Model name	Predicted value	Unit
Myricetin	VDss (human)	1.317	log L/kg
	Fraction unbound (human)	0.238	Fu
	BBB permeability	-1.493	log BB
	CNS permeability	-3.709	log PS

4.12.3 Metabolic Properties of Ligand

Metabolism transforms drugs in the body, mainly in the liver, through enzymatic reactions, especially involving cytochrome P450 enzymes. It can activate, deactivate, or make drugs more water-soluble for excretion.

Phase I reactions alter the drug's structure, while Phase II reactions conjugate it to increase solubility. Metabolism affects drug efficacy and safety, with factors like genetics and diet influencing rates. Tools like pkCSM predict metabolic stability and interactions, aiding in drug optimization.

For protocatechuic acid, it is neither a substrate nor an inhibitor for CYP2D6, CYP3A4, CYP1A2, CYP2C19, or CYP2C9. Metabolic properties of ligand shows in table 4.12 (1a).

TABLE 4.12: Metabolic properties of ligand (1a)

Ligands name	Model name	Predicted value	Unit
Protocatechuic acid	CYP2D6 substrate	No	Yes/No
	CYP3A4 substrate	No	Yes/No
	CYP1A2 inhibitor	No	Yes/No
	CYP2C19 inhibitor	No	Yes/No
	CYP2C9 inhibitor	No	Yes/No
	CYP2D6 inhibitor	No	Yes/No
	CYP3A4 inhibitor	No	Yes/No

For syringic acid, it is neither a substrate nor an inhibitor for CYP2D6, CYP3A4, CYP1A2, CYP2C19, or CYP2C9. Metabolic properties of ligand shows in table 4.13 (1b).

TABLE 4.13: Metabolic properties of ligand (1b)

Ligands name	Model name	Predicted value	Unit
Syringic acid	CYP2D6 substrate	No	Yes/No
	CYP3A4 substrate	No	Yes/No
	CYP1A2 inhibitor	No	Yes/No
	CYP2C19 inhibitor	No	Yes/No
	CYP2C9 inhibitor	No	Yes/No

Table 4.13 continued from previous page

Ligands name	Model name	Predicted value	Unit
	CYP2D6 inhibitor	No	Yes/No
	CYP3A4 inhibitor	No	Yes/No

For p-coumaric acid, it is neither a substrate nor an inhibitor for CYP2D6, CYP3A4, CYP1A2, CYP2C19, or CYP2C9. Metabolic properties of ligand shows in table 4.14 (1c).

TABLE 4.14: Metabolic properties of ligand (1c)

Ligands name	Model name	Predicted value	Unit
p-Coumaric acid	CYP2D6 substrate	No	Yes/No
	CYP3A4 substrate	No	Yes/No
	CYP1A2 inhibitor	No	Yes/No
	CYP2C19 inhibitor	No	Yes/No
	CYP2C9 inhibitor	No	Yes/No
	CYP2D6 inhibitor	No	Yes/No
	CYP3A4 inhibitor	No	Yes/No

For vanillic acid, it is neither a substrate nor an inhibitor for CYP2D6, CYP3A4, CYP1A2, CYP2C19, or CYP2C9. Metabolic properties of ligand shows in table 4.15(1d).

TABLE 4.15: Metabolic properties of ligand (1d)

Ligands name	Model name	Predicted value	Unit
Vanillic acid	CYP2D6 substrate	No	Yes/No
	CYP3A4 substrate	No	Yes/No
	CYP1A2 inhibitor	No	Yes/No
	CYP2C19 inhibitor	No	Yes/No
	CYP2C9 inhibitor	No	Yes/No
	CYP2D6 inhibitor	No	Yes/No
	CYP3A4 inhibitor	No	Yes/No

For myricetin, it is neither a CYP1A2 inhibitor but neither a substrate nor an inhibitor for CYP2D6, CYP3A4, CYP2C19, or CYP2C9. Metabolic properties of ligand shows in table 4.16 (1e).

TABLE 4.16: Metabolic properties of ligand (1e)

Ligands name	Model name	Predicted value	Unit
Myricetin	CYP2D6 substrate	No	Yes/No
	CYP3A4 substrate	No	Yes/No
	CYP1A2 inhibitor	Yes	Yes/No
	CYP2C19 inhibitor	No	Yes/No
	CYP2C9 inhibitor	No	Yes/No
	CYP2D6 inhibitor	No	Yes/No
	CYP3A4 inhibitor	No	Yes/No

4.12.4 Excretion Properties of Ligand

Excretion removes drugs and their metabolites from the body, primarily through the kidneys (urine) and liver (bile and feces). It also occurs via the lungs, skin, and breast milk. Key excretion properties, like total clearance and renal substrate status, help predict drug action duration and potential toxicity. Tools like pkCSM predict these properties, aiding in optimizing drugs for safe elimination. The excretion properties of the ligands are as follows: Protocatechuic acid has a total clearance of 0.551 log ml/min/kg and is not a renal OCT2 substrate. Syringic acid shows a total clearance of 0.646 log ml/min/kg, with no interaction with renal OCT2. p-Coumaric acid has a total clearance of 0.662 log ml/min/kg and is also not a renal OCT2 substrate. Vanillic acid's total clearance is 0.628 log ml/min/kg, and it does not interact with renal OCT2. Myricetin has the lowest total clearance at 0.422 log ml/min/kg and is not a renal OCT2 substrate. Excretion properties of ligand shows in table 4.17(1a).

TABLE 4.17: Excretion properties of ligand (1a)

Ligands Names	Model Name	Predicted Value	Unit
Protocatechuic acid	Total Clearance	0.551	log ml/min/kg
	Renal OCT2 substrate	No	Yes/No
Syringic acid	Total Clearance	0.646	log ml/min/kg
	Renal OCT2 substrate	No	Yes/No
p-Coumaric acid	Total Clearance	0.662	log ml/min/kg
	Renal OCT2 substrate	No	Yes/No

Table 4.17 continued from previous page

Ligands Names	Model Name	Predicted Value	Unit
Vanillic acid	Total Clearance	0.628	log ml/min/kg
	Renal OCT2 substrate	No	Yes/No
Myricetin	Total Clearance	0.422	log ml/min/kg
	Renal OCT2 substrate	No	Yes/No

4.12.5 Toxicity Properties of Ligand

Evaluating toxicity is vital in drug development to identify potential adverse effects. Key endpoints include AMES toxicity, MTD, and various organ toxicities. Positive toxicity results highlight risks, guiding dose selection and risk management. Early understanding of a drug's toxicity helps optimize safety. Tools like pkCSM assist in predicting toxicity to ensure safer and more effective drugs.

For protocatechuic acid, the toxicity properties are as follows: It is not AMES toxic, a hepatotoxicity, or a skin sensitizer. It is not an hERG I or II inhibitor. The maximum tolerated dose (MTD) is 0.814 log mg/kg/day. Oral rat acute toxicity (LD50) is 2.423 mol/kg, and chronic toxicity (LOAEL) is 2.021 log mg/kg_bw/day. Toxicity to *T. pyriformis* is 0.273 log μg/L, and minnow toxicity is 2.451 log mM. Toxicity properties of ligand shows in table 4.18 (1a).

Table 4.18: Toxicity properties of ligand (1a)

Ligands name	Model name	Predicted value	Unit
Protocatechuic acid	AMES toxicity	No	Yes/No
	Max. tolerated dose (human)	0.814	log mg/kg/day
	hERG I inhibitor	No	Yes/No
	hERG II inhibitor	No	Yes/No
	Oral Rat Acute Toxicity (LD50)	2.423	mol/kg
	Oral Rat Chronic Toxicity (LOAEL)	2.021	log mg/kg_bw/day
	Hepatotoxicity	No	Yes/No
	Skin Sensitisation	No	Yes/No

Table 4.18 continued from previous page

Ligands name	Model name	Predicted value	Unit
	T.Pyriformis toxicity	0.273	log ug/L
	Minnow toxicity	2.451	log mM

For syringic acid, the toxicity properties are as follows: It is not AMES toxic, nor an hERG I or II inhibitor, and does not cause hepatotoxicity or skin sensitization. The maximum tolerated dose (MTD) is 1.374 log mg/kg/day.

Oral rat acute toxicity (LD50) is 2.157 mol/kg, and chronic toxicity (LOAEL) is 2.415 log mg/kg_bw/day. Toxicity to *T. pyriformis* is 0.281 log μg/L, and minnow toxicity is 2.554 log mM. Toxicity properties of ligand shows in table 4.19 (1b)

TABLE 4.19: Toxicity properties of ligand (1b)

Ligands name	Model name	Predicted value	Unit
Syringic acid	AMES toxicity	No	Yes/No
	Max. tolerated dose (human)	1.374	log mg/kg/day
	hERG I inhibitor	No	Yes/No
	hERG II inhibitor	No	Yes/No
	Oral Rat Acute Toxicity (LD50)	2.157	mol/kg
	Oral Rat Chronic Toxicity (LOAEL)	2.415	log mg/kg_bw/day
	Hepatotoxicity	No	Yes/No
	Skin Sensitisation	No	Yes/No
	T.Pyriformis toxicity	0.281	log ug/L
	Minnow toxicity	2.554	log mM

For p-coumaric acid, the toxicity properties are as follows: It is not AMES toxic, nor an hERG I or II inhibitor, and does not cause hepatotoxicity or skin sensitization. The maximum tolerated dose (MTD) is 1.111 log mg/kg/day.

Oral rat acute toxicity (LD50) is 2.155 mol/kg, and chronic toxicity (LOAEL) is 2.534 log mg/kg_bw/day. Toxicity to *T. pyriformis* is 0.319 log μg/L, and minnow toxicity is 1.607 log mM. Toxicity properties of ligand shows in table 4.20 (1c).

TABLE 4.20: Toxicity properties of ligand (1c)

Ligands name	Model name	Predicted value	Unit
p-Coumaric acid	AMES toxicity	No	Yes/No
	Max. tolerated dose (human)	1.111	log mg/kg/day
	hERG I inhibitor	No	Yes/No
	hERG II inhibitor	No	Yes/No
	Oral Rat Acute Toxicity (LD50)	2.155	mol/kg
	Oral Rat Chronic Toxicity (LOAEL)	2.534	log mg/kg_bw/day
	Hepatotoxicity	No	Yes/No
	Skin Sensitisation	No	Yes/No
	T.Pyriformis toxicity	0.319	log ug/L
	Minnow toxicity	1.607	log mM

For vanillic acid, the toxicity properties are as follows: It is not AMES toxic, nor an hERG I or II inhibitor, and does not cause hepatotoxicity or skin sensitization. The maximum tolerated dose (MTD) is 0.719 log mg/kg/day. Oral rat acute toxicity (LD50) is 2.454 mol/kg, and chronic toxicity (LOAEL) is 2.032 log mg/kg_bw/day. Toxicity to *T. pyriformis* is 0.265 log μg/L, and minnow toxicity is 1.926 log mM. Toxicity properties of ligand shows in table 4.21 (1d).

TABLE 4.21: Toxicity properties of ligand (1d)

Ligands name	Model name	Predicted value	Unit
Vanillic acid	AMES toxicity	No	Yes/No
	Max. tolerated dose (human)	0.719	log mg/kg/day
	hERG I inhibitor	No	Yes/No
	hERG II inhibitor	No	Yes/No
	Oral Rat Acute Toxicity (LD50)	2.454	mol/kg
	Oral Rat Chronic Toxicity (LOAEL)	2.032	log mg/kg_bw/day
	Hepatotoxicity	No	Yes/No
	Skin Sensitisation	No	Yes/No

Table 4.21 continued from previous page

Ligands name	Model name	Predicted value	Unit
	T.Pyriformis toxicity	0.265	log ug/L
	Minnow toxicity	1.926	log mM

For myricetin, the toxicity properties are as follows: It is not AMES toxic, nor an hERG I or II inhibitor, and does not cause hepatotoxicity or skin sensitization. The maximum tolerated dose (MTD) is 0.51 log mg/kg/day. Oral rat acute toxicity (LD50) is 2.497 mol/kg, and chronic toxicity (LOAEL) is 2.718 log mg/kg_bw/day. Toxicity to *T. pyriformis* is 0.286 log μg/L, and minnow toxicity is 5.023 log mM. Toxicity properties of ligand shows in table 4.22(1e).

TABLE 4.22: Toxicity properties of ligand (1e)

Ligands name	Model name	Predicted value	Unit
Myricetin	AMES toxicity	No	Yes/No
	Max. tolerated dose (human)	0.51	log mg/kg/day
	hERG I inhibitor	No	Yes/No
	hERG II inhibitor	No	Yes/No
	Oral Rat Acute Toxicity (LD50)	2.497	mol/kg
	Oral Rat Chronic Toxicity (LOAEL)	2.718	log mg/kg_bw/day
	Hepatotoxicity	No	Yes/No
	Skin Sensitisation	No	Yes/No
	T.Pyriformis toxicity	0.286	log ug/L
	Minnow toxicity	5.023	log mM

4.13 Lipinski's Rule of Five

The next step in our analysis involves applying Lipinski's Rule of Five, a widely used guideline in drug discovery, to assess the drug-likeness of the selected ligands. Lipinski's Rule of Five evaluates key physicochemical properties of compounds to

predict their likelihood of being orally bioavailable and pharmacologically active. Here's how the rule was applied to our ligands:

1. **Molecular weight:** (under 500 Daltons)
2. **logP:** (less than 5)
3. **Hydrogen bond donors:** (no more than 5)
4. **Hydrogen bond acceptors:** (no more than 10)
5. **Number of rotatable bonds:** (no more than 5)

This table 4.23 details the physicochemical properties of various ligands, including molecular weight, logP, number of rotatable bonds, hydrogen bond acceptors and donors, and surface area.

TABLE 4.23: Physicochemical Properties of Ligands

Ligands	Molecular weight	logP	Rotatable bonds	Acceptors	Donors	Surface area
Protocatechuic acid	154.121	0.796	1	3	3	62.341
Syringic acid	198.174	1.1076	3	4	2	80.53
p-Coumaric acid	164.16	1.49	2	2	2	69.587
Vanillic acid	168.148	1.099	2	3	2	69.025
Myricetin	318.237	1.6936	1	8	6	126.902

Lipinski's Rule of Five states that most compounds with no more than one violation of these rules are likely to have good oral bioavailability.

After assessing each ligand against Lipinski's Rule of Five, we determined their drug-likeness and potential for oral bioavailability. Ligands that adhere to Lipinski's rule are generally more favorable for further drug development due to their increased probability of success in terms of absorption and pharmacological activity.

4.14 Utilizing CB Dock 2 for Molecular Docking

To perform molecular docking simulations, it's crucial to prepare the protein and ligand files appropriately. The ligand structures were saved in SDF format, which allows for the representation of molecular structures with atom-by-atom detail. On the other hand, the protein structure was saved in PDB format, a standard file format used for representing 3D structures of proteins and other macromolecules.

In CB Dock 2, these refined protein and ligand files were uploaded without any gaps or errors in their filenames. CB Dock 2 is molecular docking software that predicts the preferred orientation of a ligand when bound to a protein receptor. By analyzing various conformations and orientations, it helps in predicting the most energetically favorable binding mode between the ligand and the protein.

The protein structure, refined and optimized using PyMOL, provides the receptor for ligand binding, while the ligand structures represent potential drug candidates. CB Dock 2 uses algorithms to explore the binding interactions between the ligands and the protein's active sites, predicting their binding affinities and orientations.

By using CB Dock 2, researchers can gain insights into how the ligands interact with the protein target, helping in the identification of potential lead compounds for further development as drugs or therapeutics.

4.14.1 Docking complex of Protocatechuic Acid

In the CB-Dock 2 molecular docking analysis, Protocatechuic acid with a Vina score of -4.3, demonstrates a solid binding affinity with the target protein. The binding cavity's volume of 232 A^3 indicates that the ligand fits well within the docking site, and the dimensions of the docking box ($17 \times 17 \times 17$ A^3) are appropriately sized for effective docking. Figure 4.9 shows the docking complex of protocatechuic acid.

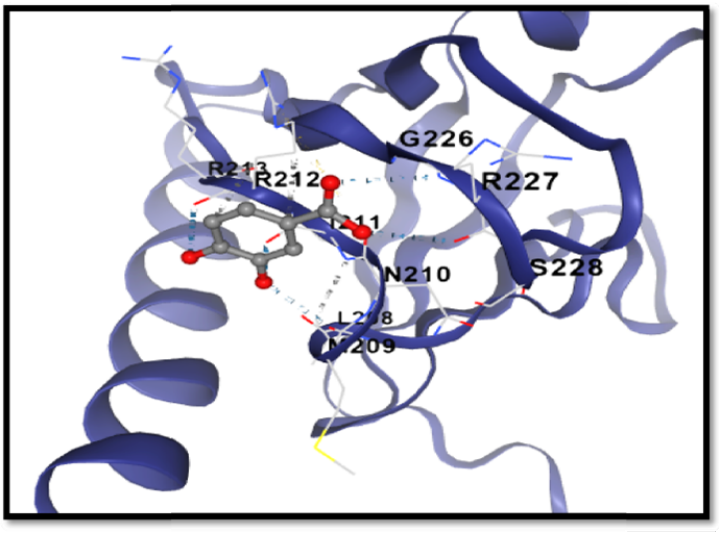


FIGURE 4.9: Docking complex of Protocatechuic acid

4.14.2 Docking complex of Syringic acid

Syringic acid has a Vina score of -4.6, which indicates a better predicted binding affinity. This score suggests that Syringic acid may bind more effectively to the target protein. The binding cavity volume of 67 A^3 is relatively smaller, which can mean a tighter and potentially more specific interaction with the ligand. The docking box size ($17 \times 17 \times 17$ A^3) is appropriate for the docking process. Figure 4.10 shows the docking complex of syringic acid.

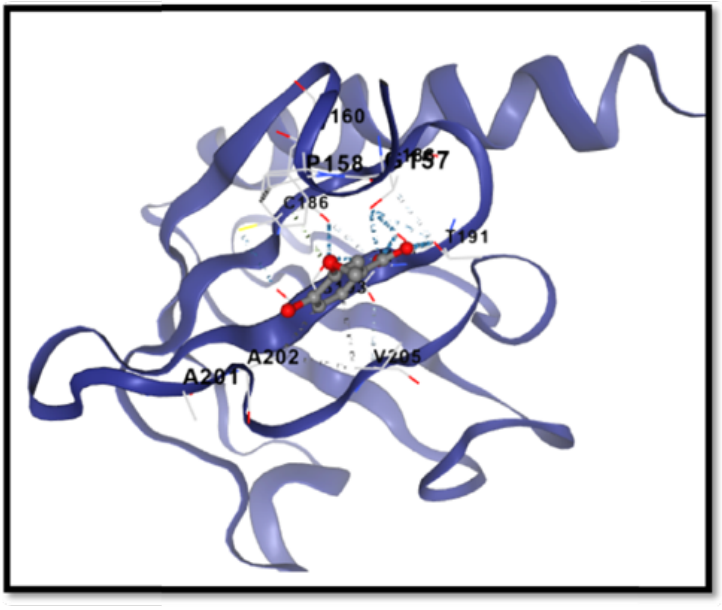


FIGURE 4.10: Docking complex of Syringic acid

4.14.3 Docking complex of p-Coumaric acid

p-Coumaric acid has a Vina score of -4.6, indicating a binding affinity similar to Syringic acid. This suggests that p-Coumaric acid binds effectively to the target protein. The binding cavity volume is 92 A^3, which is larger than that of Syringic acid, potentially allowing for some flexibility in the binding interactions. The docking box size is 18 × 18 × 18 A^3, providing adequate space for docking. Figure 4.11 shows the docking complex of p-Coumaric acid.

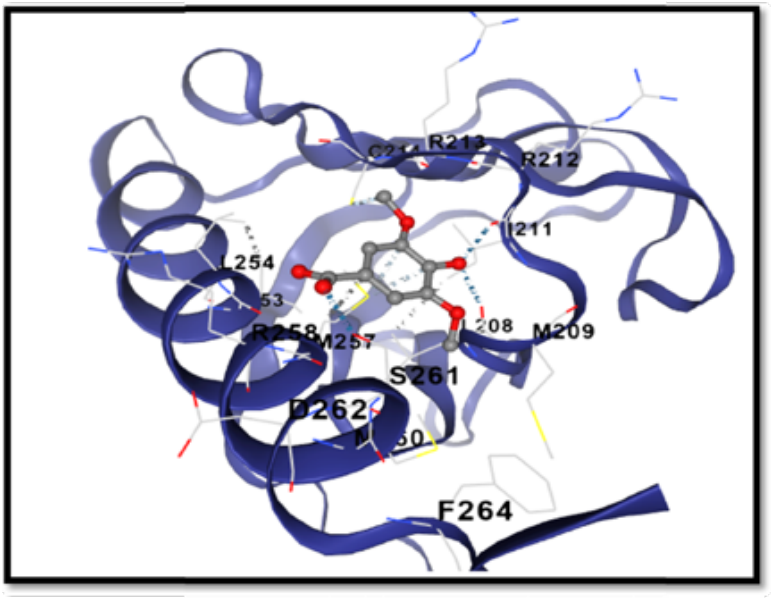


FIGURE 4.11: Docking complex of p-Coumaric acid

4.15 Docking Complex of Vanillic acid

Vanillic acid has a Vina score of -4.9, indicating the highest predicted binding affinity among the ligands listed, suggesting it binds quite effectively to the target protein. The binding cavity volume is 67 A^3, which is the same as Syringic acid, implying a similar size of the binding site. The docking box size is 19 × 19 × 19 A^3, providing ample space for the docking process. Figure 4.12 shows the docking complex of vanillic acid.

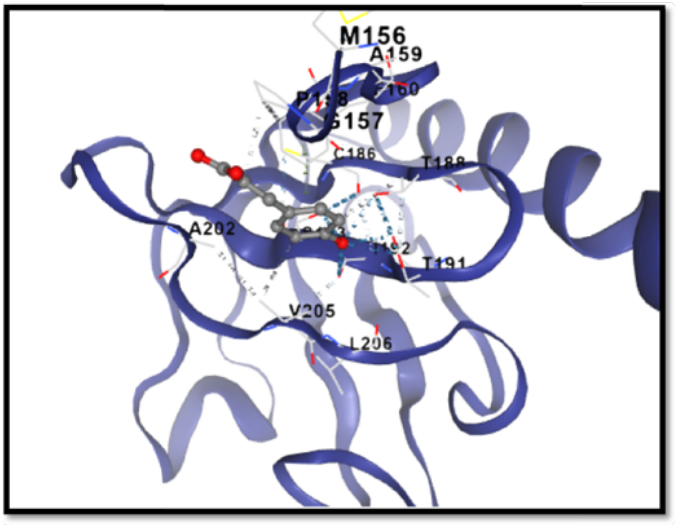


FIGURE 4.12: Docking complex of Vanillic acid

4.15.1 Docking complex of Myricetin

Myricetin has a Vina score of -6.1, which is the most negative among the listed ligands, indicating the highest predicted binding affinity to the target protein. This suggests that Myricetin is likely to bind most effectively compared to the other compounds. The binding cavity volume is 92 A^3, similar to p-Coumaric acid, allowing for a potentially flexible binding interaction. The docking box size is 21 × 21 × 21 A^3, providing ample space for the docking process. Figure 4.13 shows the docking complex of myricetin.

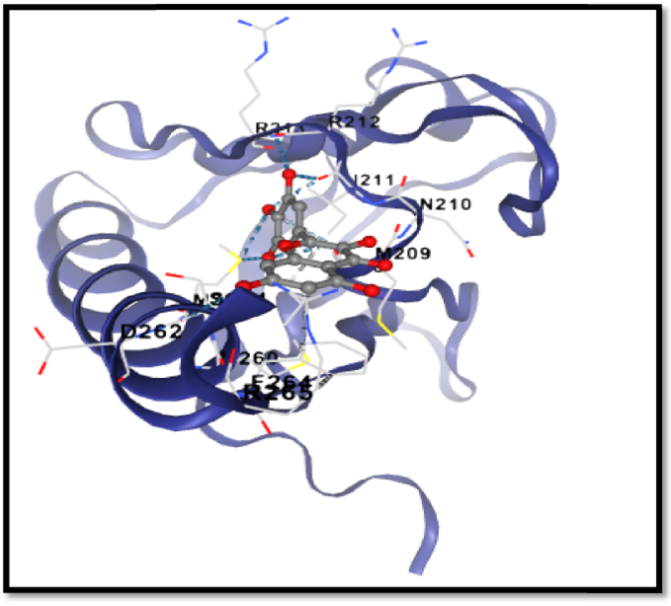


FIGURE 4.13: Docking complex of Myricetin

This table 4.24 presents the docking properties of various ligands, including Vina scores, cavity volumes, center coordinates, and docking sizes.

TABLE 4.24: Docking Properties of Ligands

Ligands name	Vina Score	Cavity volume (A^3)	Center (x, y, z)	Docking size (x, y, z)
Protocatechuic acid	-4.3	232	5, -2, 12	17, 17, 17
Syringic acid	-4.6	67	2, 10, 8	17, 17, 17
p-Coumaric acid	-4.6	92	-8, -1, -6	18, 18, 18
Vanillic acid	-4.9	67	2, 10, 8	19, 19, 19
Myricetin	-6.1	92	-8, -1, -6	21, 21, 21

4.16 Analyzing Docking Results Using LigPlot

After conducting molecular docking simulations using CB Dock 2, the docking results will be obtained, showing potential binding interactions between ligands and the protein receptor. To analyze these interactions, LigPlot will be used. LigPlot is a tool that generates 2D diagrams illustrating the interactions between ligands and proteins, focusing on polar bonds, hydrogen bonds, and hydrophobic interactions.

The analysis of docking results will first involve examining the potential binding poses of ligands within the protein's active site, as obtained from CB Dock 2. These poses will provide detailed information about hydrogen bonding, hydrophobic interactions, and other intermolecular contacts crucial for ligand binding. Subsequently, LigPlot will be used to visualize these interactions, generating schematic 3D diagrams that highlight polar bonds, hydrogen bonds, and hydrophobic interactions between the ligands and protein amino acid residues. Identifying polar bonds is particularly important, as these interactions significantly influence the ligand's binding affinity and specificity. By analyzing these polar interactions, we can evaluate their strength and significance in stabilizing the ligand-protein

complex. This comprehensive analysis will offer insights into the key residues involved in ligand binding and deepen our understanding of the molecular basis of ligand-receptor interactions.

By employing LigPlot to analyze the docking results, we can gain a deeper understanding of the specific interactions driving ligand binding to the protein target, facilitating the rational design and optimization of ligands for improved therapeutic efficacy.

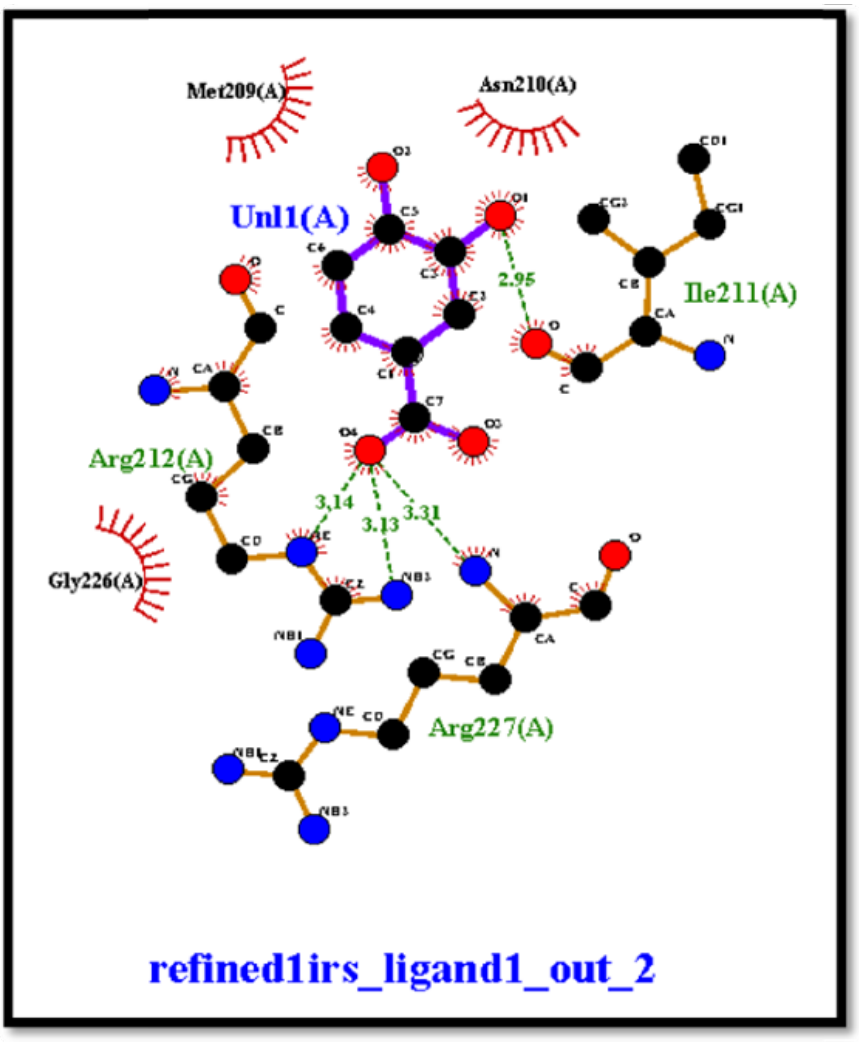


FIGURE 4.14: Interaction of Protocatechuic acid

Figure 4.14 shows the interaction of Protocatechuic acid with receptor protein. It shows that Protocatechuic acid has formed three hydrophobic interactions and four hydrogen bonds.

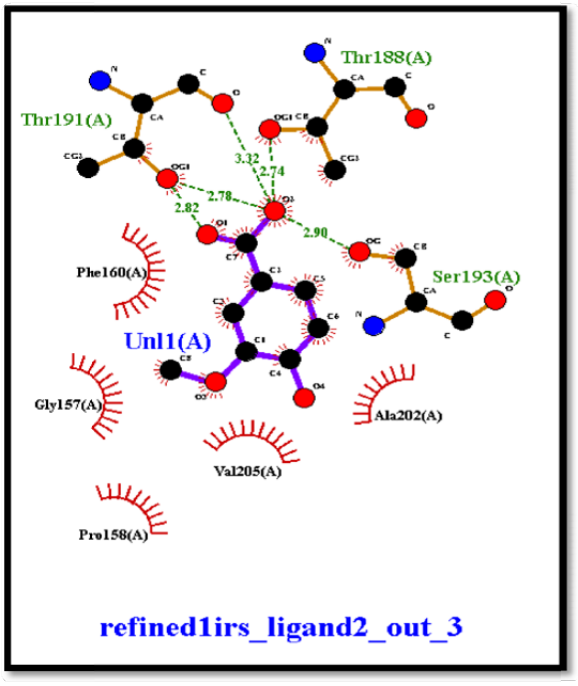


FIGURE 4.15: Interaction of Syringic acid

Figure 4.15 shows the interaction of syringic acid with receptor protein. It shows that syringic acid has formed five hydrophobic interactions and five hydrogen bonds.

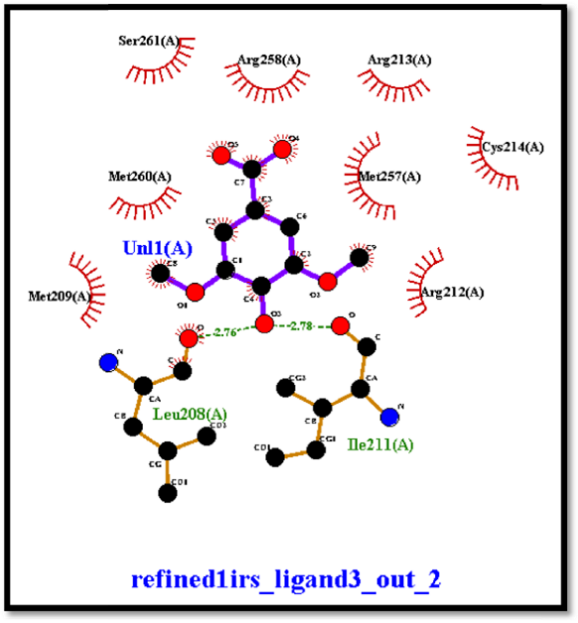


FIGURE 4.16: Interaction of p-Coumaric acid

Figure 4.16 shows the interaction of p-Coumaric acid with receptor protein. It shows that p- Coumaric acid has formed eight hydrophobic interactions and two hydrogen bonds.

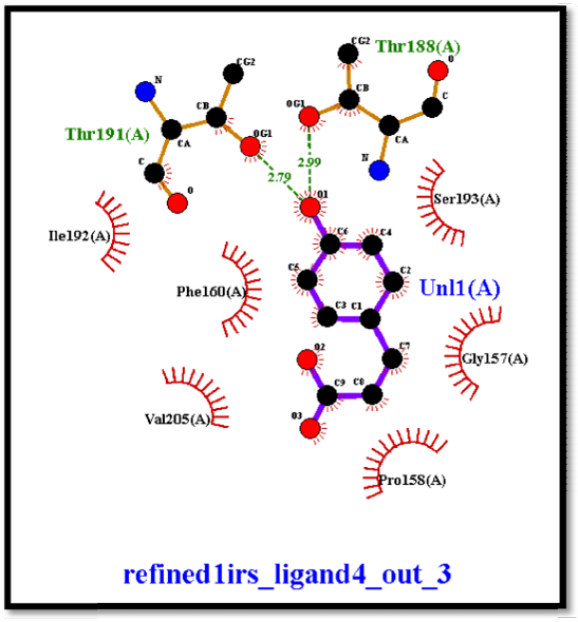


FIGURE 4.17: Interaction of Vanillic acid

Figure 15 shows the interaction of vanillic acid with receptor protein. It shows that vanillic acid has formed six hydrophobic interactions and two hydrogen bonds.

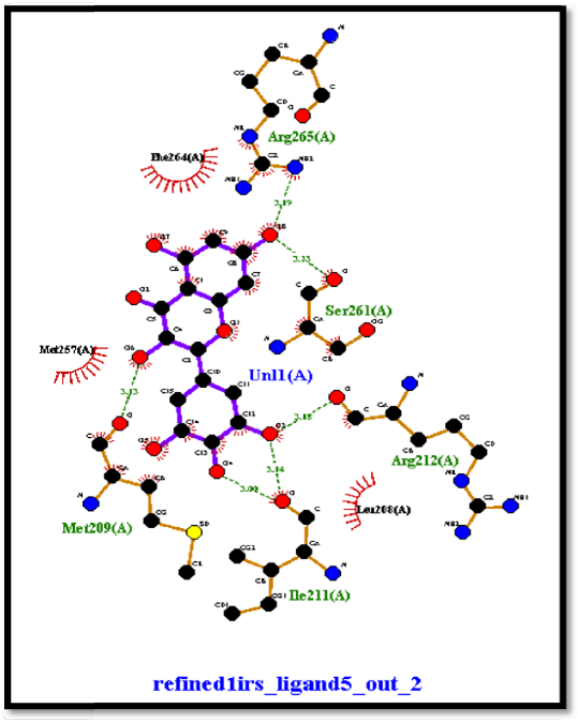


FIGURE 4.18: Interaction of Myricetin

Figure 4.18 shows the interaction of myricetin with receptor protein. It shows that myricetin has formed three hydrophobic interactions and six hydrogen bonds.

Properties of compounds obtained from ligplot such as amino acid, hydrogen bonding and hydrophobic bonding shows in table 4.25.

TABLE 4.25: Interaction Properties of Compounds as Visualized by LigPlot

Ligand Names	Amino Acids	H Bonding Distance	Hydrophobic Interaction
Protocatechuric acid	1. Ile211	2.95	Met209
	2. Arg212	3.13	Asn210
	3. Arg227	3.14	Gly226
		3.31	
Syringic acid	1. Thr188	2.74	Phe160
	2. Thr191	2.78	Pro158
	3. Ser193	2.82	Gly157
		2.9	Val205
		3.32	Ala202
p-coumaric acid	1. Leu208	2.76	Ser261
	2. lle211	2.78	Arg213
			Arg258
			Met257
			Met260
			Arg212
			Cys214
			Met209
Vanillic acid	1. Thr191	2.79	lle192
	2. Thr188	2.99	Pro158
			Phe160
			Val205
			Ser193
			Gly157
Myricetin	1. Arg265	3	Ehe264
	2. Ser261	3.13	Met257
	3. Arg212	3.14	Leu208
	4. Met209	3.18	
	5. lle211	3.19	
		3.23	

4.17 Lead Compound Identification

Protocatechuic acid was identified as a lead compound through a rigorous screening process that evaluates physicochemical properties, binding scores, ADME/T properties, and compliance with the Lipinski Rule of Five.

4.18 Reference Anti-Diabetic Drug Identification

The selection of the most efficient anti-diabetic drug is based on its physicochemical and ADME/T properties, along with its mechanism of action and side effects. The physicochemical properties are retrieved from the PubChem online database, while the ADME/T properties are assessed using the pkCSM online tool. The mechanism of action is identified through the DrugBank and KEGG.

4.19 Metformin Mechanism of Action

Metformin works by reducing hepatic glucose production and increasing insulin sensitivity. It activates AMP-activated protein kinase (AMPK), which inhibits gluconeogenic enzymes, decreasing glucose production in the liver. Additionally, Metformin enhances glucose uptake in muscle and fat tissues by promoting GLUT4 translocation to the cell membrane. It may also reduce intestinal glucose absorption and alter gut microbiota, contributing to its glucose-lowering

effect. Metformin is effective in lowering blood glucose levels without significant hypoglycemia, though it can cause gastrointestinal side effects and, rarely, lactic acidosis. Metformin's effective mechanism of action and favorable safety profile make it an ideal reference drug for comparative studies, providing a solid foundation for evaluating new potential therapeutic agents such as Protocatechuic acid in the context of IRS protein sensitivity and glucose metabolism [101].

4.20 Chemical Structure of Metformin

The chemical structure is characterized by a simple yet effective design, which is pivotal to its function. The molecule comprises a biguanide group a central feature that consists of two guanidine groups linked by a single carbon atom. This structure is represented by its IUPAC name, 1,1-Dimethylimidazolidine-2,4-dione. The biguanide moiety is crucial for its mechanism of action, which primarily involves decreasing hepatic glucose production and enhancing insulin sensitivity. Metformin's straightforward structure allows it to effectively interact with cellular targets, contributing to its efficacy as a first-line treatment for type 2 diabetes.

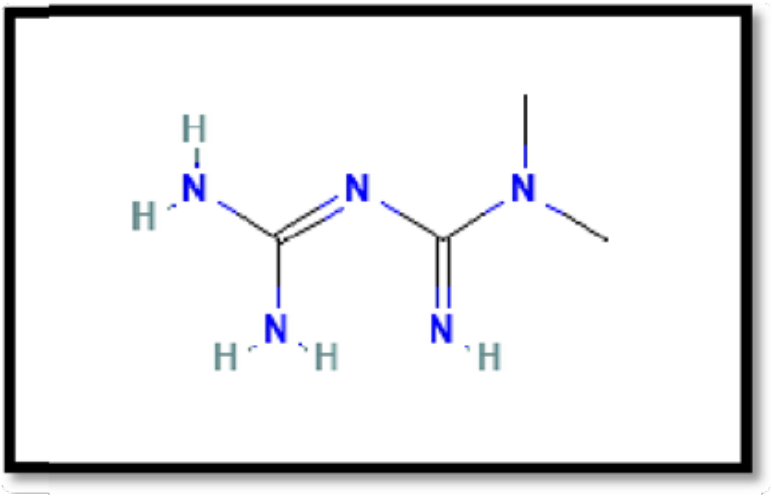


FIGURE 4.19: Chemical structure of Metformin

4.21 Energy Minimization of Metformin

The energy minimization of metformin revealed a dipole-dipole interaction energy of -7.8627 kcal/mol and a total energy of 11.0122 kcal/mol. These values serve as a comparison for protocatechuic acid, the lead compound in the research, helping to assess their relative stability and interactions.

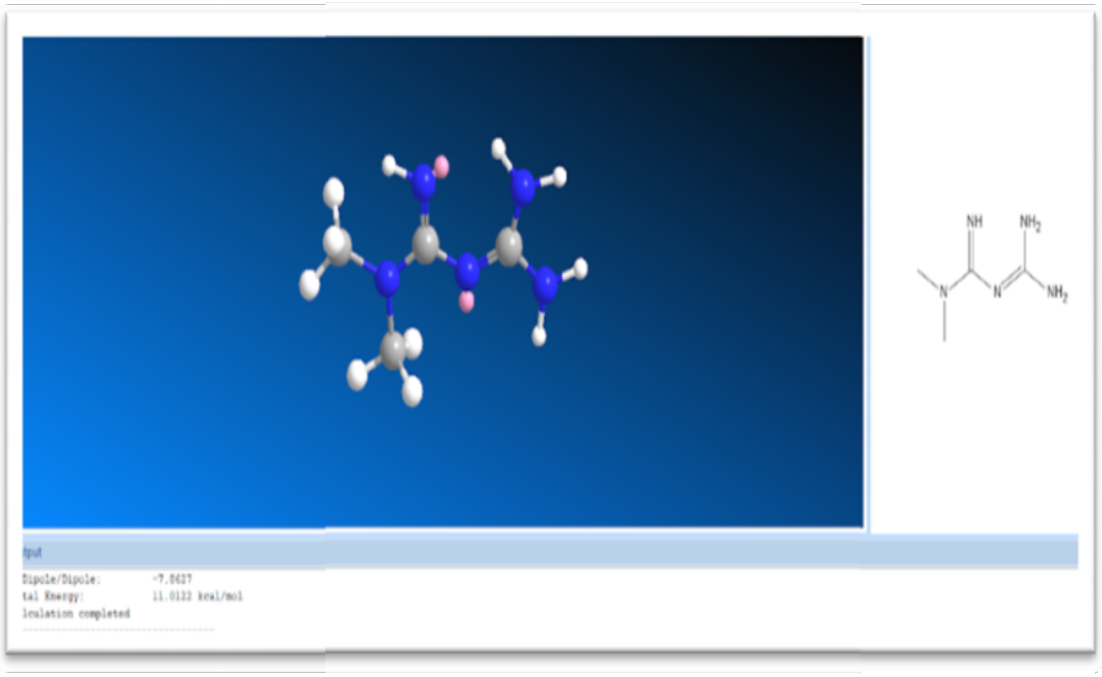


FIGURE 4.20: Energy minimization of metformin

4.22 Drug ADME/T Properties Comparison

ADME/T properties of FDA approved drug were explored by pkCSM online prediction tool.

4.22.1 Absorption Properties Comparison

The comparison of absorption properties indicates that protocatechuic acid has better water solubility, higher CaCO2 permeability, and higher predicted intestinal absorption compared to metformin. This suggests that protocatechuic acid might have superior absorption characteristics, which could enhance its bioavailability. Additionally, the lack of interaction with P-glycoprotein for protocatechuic acid could result in fewer drug-drug interactions and a different pharmacokinetic profile compared to metformin. These properties make protocatechuic acid a promising lead compound for further investigation in the context of improving IRS1 protein sensitivity. Absorption properties of drug and lead compound shows in table 4.26 (1a).

TABLE 4.26: Absorption properties of drug and lead compound (1a)

Ligands name	Model name	Predicted value	Unit
Metformin	Water solubility	-2.707	log mol/L
	Caco2 permeability	-0.339	log Papp in 10-6 cm/s
	Intestinal absorption (human)	59.401	(% Absorbed)
	Skin Permeability	-2.735	Log Kp
	P-glycoprotein substrate	Yes	Yes/No
	P-glycoprotein I inhibitor	No	Yes/No
	P-glycoprotein II inhibitor	No	Yes/No
Protocatechuic acid	Water solubility	-2.069	log mol/L
	Caco2 permeability	0.49	log Papp in 10-6 cm/s
	Intestinal absorption (human)	71.174	(% Absorbed
	Skin Permeability	-2.727	log Kp
	P-glycoprotein substrate	No	Yes/No
	P-glycoprotein I inhibitor	No	Yes/No
	P-glycoprotein II inhibitor	No	Yes/No

4.22.2 Distribution Properties Comparison

The comparison of distribution properties indicates that metformin has a higher volume of distribution and fraction unbound in plasma, suggesting a greater extent of tissue distribution and availability for pharmacological action. Protocatechuic acid, on the other hand, shows a lower volume of distribution, indicating it remains more within the systemic circulation.

While both compounds have low BBB and CNS permeability, protocatechuic acid has slightly better values in these aspects, suggesting it may have a marginally better ability to affect the CNS if needed.

These distribution characteristics highlight differences in how these compounds might behave in the body, which can influence their therapeutic applications and potential side effects. Distribution properties of drug and lead compound in table 4.27 (1b).

TABLE 4.27: Distribution properties of drug and lead compound (1b)

Ligands name	Model name	Predicted value	Unit
Metformin	VDss (human)	.0.232	log L/kg
	Fraction unbound (human)	0.811	Fu
	BBB permeability	-0.946	log BB
	CNS permeability	-4.238	log PS
Protocatechuic acid	VDss (human)	-1.298	log L/kg
	Fraction unbound (human)	0.648	Fu
	BBB permeability	-0.683	log BB
	CNS permeability	-3.305	log PS

4.22.3 Metabolism Properties Comparison

The comparison of metabolic properties reveals that both metformin and protocatechuic acid do not interact with major CYP enzymes either as substrates or inhibitors. This implies a low potential for drug-drug interactions related to the metabolism of these compounds, which is advantageous for their use as therapeutic agents. Since neither compound is metabolized by or inhibits these key enzymes, they are likely to have stable metabolic profiles, reducing the risk of adverse interactions with other medications processed by these enzymes. This metabolic stability makes protocatechuic acid a promising candidate for further development, alongside metformin, in the context of enhancing IRS protein sensitivity. Metabolic properties of drug and lead compound in table 4.28 (1c).

TABLE 4.28: Metabolic properties of drug and lead compound (1c)

Ligands name	Model name	Predicted value	Unit
Metformin	CYP2D6 substrate	No	Yes/No
	CYP3A4 substrate	No	Yes/No
	CYP1A2 inhibitor	No	Yes/No
	CYP2C19 inhibitor	No	Yes/No
	CYP2C9 inhibitor	No	Yes/No
	CYP2D6 inhibitor	No	Yes/No
	CYP3A4 inhibitor	No	Yes/No
Protocatechuic acid	CYP2D6 substrate	No	Yes/No

Table 4.28 continued from previous page

Ligands name	Model name	Predicted value	Unit
	CYP3A4 substrate	No	Yes/No
	CYP1A2 inhibitor	No	Yes/No
	CYP2C19 inhibitor	No	Yes/No
	CYP2C9 inhibitor	No	Yes/No
	CYP2D6 inhibitor	No	Yes/No
	CYP3A4 inhibitor	No	Yes/No

4.22.4 Excretion Properties Comparison

The comparison of excretion properties indicates that protocatechuic acid has a higher total clearance rate compared to metformin, suggesting it is more rapidly eliminated from the body.

The fact that neither compound is a substrate for renal OCT2 means their renal excretion pathways are unlikely to be affected by OCT2-mediated interactions. These excretion characteristics highlight potential differences in pharmacokinetics and dosing frequency between the two compounds.

Protocatechuic acid's higher clearance rate could influence its therapeutic use and necessitate adjustments in dosing to maintain effective plasma concentrations. This property, combined with its favorable absorption and distribution characteristics, continues to support its potential as a lead compound for improving IRS protein sensitivity. Metformin properties of drug and lead compound shows in table 4.29 (1d).

Table 4.29: Metformin properties of drug and lead compound (1d)

Ligands names	Model name	Predicted value	Unit
Metformin	Total Clearance	0.1	log ml/min/kg
	Renal OCT2 substrate	No	Yes/No
Protocatechuic acid	Total Clearance	0.551	log ml/min/kg
	Renal OCT2 substrate	No	Yes/No

4.22.5 Toxicity Properties Comparison

The comparison of toxicity properties reveals that protocatechuic acid has a more favorable toxicity profile compared to metformin. Protocatechuic acid is non-mutagenic, non-sensitizing to skin, and exhibits lower minnow toxicity, suggesting it may be safer for both human use and environmental impact.

While both compounds have similar profiles in terms of hepatotoxicity, hERG inhibition, and acute and chronic toxicity, the absence of mutagenicity and skin sensitization issues in protocatechuic acid strengthens its potential as a lead compound.

These favorable toxicity properties support further investigation into protocatechuic acid as a promising agent for improving IRS1 protein sensitivity. Toxicity properties of drug and lead compound shows in table 4.30 (1e).

TABLE 4.30: Toxicity properties of drug and lead compound (1e)

Ligands name	Model name	Predicted value	Unit
Metformin	AMES toxicity	Yes	Yes/No
	Max. tolerated dose (human)	0.902	log mg/kg/day
	hERG I inhibitor	No	Yes/No
	hERG II inhibitor	No	Yes/No
	Oral Rat Acute Toxicity (LD50)	2.453	mol/kg
	Oral Rat Chronic Toxicity (LOAEL)	2.158	log mg/kg_bw/day
	Hepatotoxicity	No	Yes/No
	Skin Sensitisation	Yes	Yes/No
	T.Pyriformis toxicity	0.25	log ug/L
	Minnow toxicity	3.972	log mM
Protocatechuic acid	AMES toxicity	No	Yes/No
	Max. tolerated dose (human)	0.814	log mg/kg/day
	hERG I inhibitor	No	Yes/No
	hERG II inhibitor	No	Yes/No
	Oral Rat Acute Toxicity (LD50)	2.423	mol/kg

Table 4.30 continued from previous page

Ligands name	Model name	Predicted value	Unit
	Oral Rat Chronic Toxicity (LOAEL)	2.021	log mg/kg_bw/day
	Hepatotoxicity	No	Yes/No
	Skin Sensitisation	No	Yes/No
	T.Pyriformis toxicity	0.273	log ug/L
	Minnow toxicity	2.451	log mM

4.23 Lipinski Rule of Five Comparison

Protocatechuic acid presents several advantages over metformin when evaluated against the Lipinski Rule of Five criteria. Although both compounds fall within acceptable limits for drug- like properties, protocatechuic acid's molecular weight of 154.121 g/mol and logP of 0.796 indicate a balanced hydrophilicity and lipophilicity, which could enhance its membrane permeability and overall drug efficacy. In contrast, metformin lower molecular weight and more negative logP suggest higher water solubility but potentially limited membrane permeability. Both compounds have a comparable number of hydrogen bond acceptors and donors, with protocatechuic acid showing slightly greater surface area, which might facilitate better interactions with biological targets. The presence of one rotatable bond in protocatechuic acid compared to none in metformin adds flexibility, possibly improving its ability to bind to various targets. Overall, protocatechuic acid's balanced properties and increased flexibility position it as a potentially more effective and adaptable therapeutic agent than metformin. Table 4.31 showing properties of selected drug metformin and lead compound according to Lipinski rule of 5.

TABLE 4.31: Properties of Metformin and Protocatechuic Acid Compared to Lipinski's Rule of Five

Ligands	Molecular weight	logP	Rotatable bonds	Acceptors	Donors	Surface area
Metformin	129.167	-1.24383	0	1	3	53.854
Protocatechuic acid	154.121	0.796	1	3	3	62.341

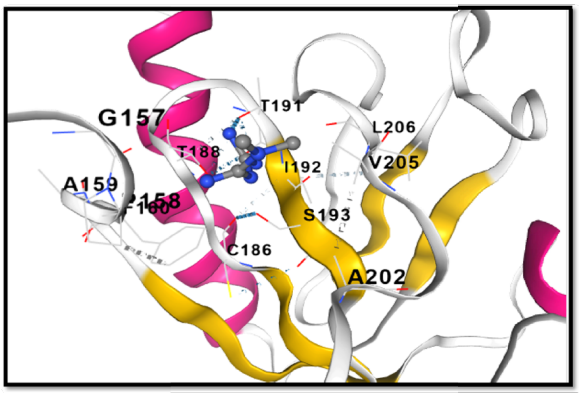


FIGURE 4.21: Docking between reference drug and IRS1

4.24 Docking Score Comparison

The docking score comparison reveals that protocatechuic acid outperforms metformin in terms of binding affinity and interaction with the target site. Protocatechuic acid achieves a Vina score of -4.3, indicating a stronger predicted binding affinity compared to metformin score of -4.0.Table 4.32 showing the docking score of lead compound and reference drug.

TABLE 4.32: Docking score of lead compound and reference drug

Compound name	Vina Score	Cavity volume (A^3)	Center (x, y, z)	Docking size (x, y, z)
Protocatechuic acid	-4.3	232	5, -2, 12	17, 17, 17
Metformin	-4	67	2, 10, 8	16,16,16

Additionally, protocatechuic acid interacts with a larger cavity volume (232 A^3) compared to metformin smaller cavity volume (67 A^3), suggesting that protocatechuic acid may occupy a more extensive binding site and potentially establish more interactions with the target. The docking center for protocatechuic acid is located at (5, -2, 12), whereas metformin binds at (2, 10, 8), with protocatechuic acid's larger docking size (17 × 17 × 17 Å) compared to metformin (16 × 16 × 16 A) supporting the potential for more robust interactions. These results highlight protocatechuic acid's superior binding characteristics, positioning it as a more promising lead compound than metformin. Figure 4.21 shows the docking between reference drug and IRS1.

4.25 Comparison Between Ligands and Target Protein

Protocatechuic acid shows a similar hydrogen bonding profile with slightly different distances and an additional interaction with Arg227, compared to metformin. The hydrogen bonding distances for protocatechuic acid are comparable to those of metformin, indicating effective binding. Protocatechuic acid also engages in additional hydrophobic interactions with Asn210 and Gly226, potentially enhancing its binding stability and interaction with the target protein. These additional interactions suggest that protocatechuic acid may have a more favorable binding profile, potentially leading to enhanced efficacy and specificity compared to metformin.

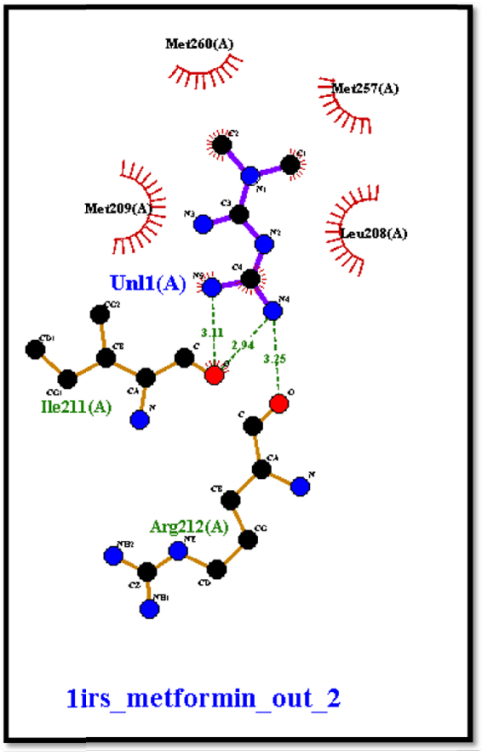


FIGURE 4.22: Interaction of IRS1 with reference drug

Figure 4.22 shows the interaction of IRS1 with reference drug. It shows that metformin has formed three hydrophobic interactions and three hydrogen bonds

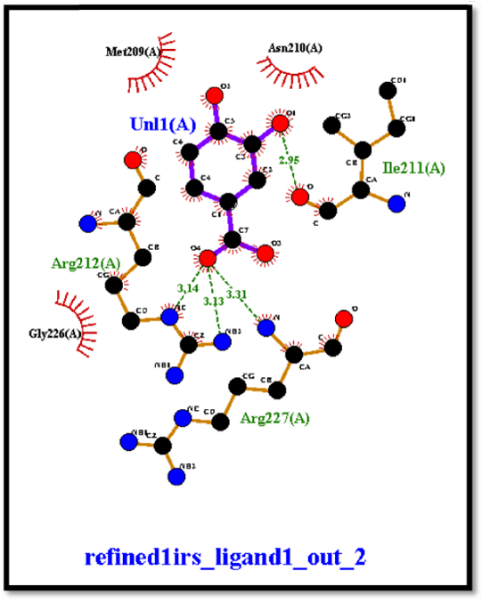


FIGURE 4.23: Interaction of Protocatechuic acid with receptor protein

Figure 4.23 shows the interaction of Protocatechuic acid with receptor protein. It shows that Protocatechuic acid has formed three hydrophobic interactions and four hydrogen bonds.

TABLE 4.33: Interaction Properties of lead compound and reference drug visualized by LigPlot

Compound Name	Amino Acids	H Bonding Distance	Hydrophobic Interaction
Metformin	1. Ile211	2.94	Leu 208
	2. Arg212	3.11	Met 209
	3. Unll	3.25	Met 257
		3.31	Met 260
Protocatechuric acid	1. Ile211	2.95	Met209
	2. Arg212	3.13	Asn210
	3. Arg227	3.14	Gly226
		3.31	

Chapter 5

Conclusion Recommendation and Limitations

This research aimed to ident fy promising compounds for treating T2D through computational methods, focusing on enhancing IRS1 sensitivity. After conducting an extensive data mining process, ten candidate ligands were selected for virtual screening. The proteins involved in this study were IRS1 and its related regulatory components. Utilizing the CB Dock automated version of AutoDock Vina for docking studies and analyzing the protein-ligand interactions with LigPlot Plus version 1.4.5, the study evaluated the binding scores, physicochemical properties, and ADME/T profiles of the selected ligands. The analysis revealed four high-potential phytocompounds: protocatechuic acid, syringic acid, p-coumaric acid, and vanillic acid. Among these, protocatechuic acid stood out as the most promising due to its superior binding affinity and favorable pharmacokinetic characteristics.

Protocatechuic acid, a bioactive compound derived from *Abelmoschus esculentus* (okra) leaves, demonstrated significant potential for modulating IRS1 sensitivity and improving insulin signaling pathways compared to the synthetic drug metformin. Its strong binding interactions with IRS1 and its favorable physicochemical and ADMET profiles suggest that it could be an effective alternative or complement to existing diabetes therapies.

Based on these findings, several recommendations are proposed. First, it is essential to conduct experimental validations through in vitro and in vivo studies to confirm the computational predictions and assess the biological activity of protocatechuic acid in modulating IRS1 sensitivity. Detailed mechanistic studies should be carried out to elucidate the specific pathways through which protocatechuic acid influences IRS1, providing insights into its therapeutic potential. Additionally, exploring the synergistic effects of protocatechuic acid with other bioactive compounds from okra leaves could enhance its therapeutic efficacy.

Extending the research to include other polyphenols and phytochemicals from okra leaves and different plant sources could uncover additional compounds with promising anti-diabetic properties. Comprehensive pharmacokinetic and safety assessments of protocatechuic acid are necessary to ensure its efficacy and address any potential safety concerns. Following promising preclinical results, initiating clinical trials will be crucial to evaluate the effectiveness and safety of protocatechuic acid in human subjects, facilitating its transition from experimental to therapeutic use.

Further investigation into the broader applications of okra leaves in traditional and modern medicine is also recommended. Given the significant pharmacological effects of protocatechuic acid, leveraging the entire plant or its extracts may offer new therapeutic options. Lastly, while the current study focuses on IRS1 modulation, future research should consider the role of quorum sensing in diabetes management, as understanding its influence could lead to new therapeutic strategies.

The research highlights the potential of protocatechuic acid as a promising therapeutic agent for T2D. Continued research, including experimental validation and clinical trials, is essential to translate these findings into effective and safe treatments. Addressing these recommendations will advance the development of novel, plant-based therapies for managing T2D and improving patient outcomes.

One of the primary limitations of this research is its insilico nature, which may not fully capture the complex physiological interactions of protocatechuic acid

within the human body. While computational tools provide valuable insights into molecular docking and protein interactions, they cannot replicate the dynamic environment of in vivo systems, where factors like bioavailability, metabolism, and potential side effects play a critical role. Additionally, the study focuses solely on the IRS1 protein and its role in insulin signaling, potentially overlooking other pathways that might contribute to the overall effect of protocatechuic acid in diabetes management. Further experimental validation through in vitro or in vivo studies is needed to confirm the computational predictions and understand the broader biological implications.

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