CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Effect of *Momordica charantia* Polyphenols on Sensitivity of IRS1 protien: An Insilico Study

by

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To my dear parents, whose unconditional love and support have been my greatest strength. Your encouragement and belief in my abilities have been the driving force behind this achievement. This work is a tribute to your endless sacrifices and unwavering faith in me.

CERTIFICATE OF APPROVAL

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Abstract

Diabetes mellitus (DM), defined by abnormal glucose metabolism and persistent hyperglycemia, poses a serious danger to global health. Pancreatic cells die autoimmunely, resulting in type 1 diabetes. whereas insulin resistance and pancreatic -cell malfunction, which are commonly aggravated by aging and lifestyle factors, are linked to type 2 diabetes. Diabetes patients with persistent hyperglycemia are more prone to experience serious consequences affecting the kidneys, heart, blood vessels, eyes, and nerves, demanding effective treatment choices.

In insulin signaling pathways, insulin receptor substrate (IRS1) proteins, particularly IRS-1 and IRS-2, interpret insulin and insulin-like growth factor (IGF) signals to control cellular growth and glucose metabolism. IRS-1 loss in mice causes severe growth retardation, demonstrating its importance in controlling organismal growth. IRS-2 deficiency, on the other hand, is caused by reduced -cell activity and results in tissue-specific abnormalities and early-onset diabetes, underscoring its importance in metabolic balance.

As per a recent report of 2023 on the counter diabetic properties of *Momordica charantia* removes, bioactive mixtures, for example, cucurbitane-type triterpenoids further developed glucose digestion and insulin responsiveness by means of various systems, including balance of insulin flagging pathways and cell reinforcement exercises.

Recent clinical trials in 2022 demonstrated that supplementation with *Momordica charantia* extracts effectively reduced fasting blood glucose levels and HbA1c in patients with type 2 diabetes, highlighting its potential as a complement to conventional diabetes medications. Nanoencapsulation, which improved the compounds' stability and absorption in diabetic animal models, was used in a 2022 study to increase the bioavailability of *Momordica charantia* bioactive components.

In 2023, comparative studies compared *Momordica charantia*'s effectiveness to that of other natural antidiabetic medications like Berberine. They found that

Momordica charantia had the same or better effects on glucose control and lipid profile management in diabetic patients.

Hereditary predisposition, as well as natural factors such as nutrition and lifestyle, have a significant impact on diabetes risk. Recent research has focused on natural chemicals found in *Momordica charantia* (bitter gourd), which contain cucurbitane-type triterpenoids believed to have the ability to lower insulin resistance. These compounds inhibit insulin resistance-related enzymes, implying potential therapeutic benefits for T2DM therapy.

Significant interactions between *Momordica charantia*'s polyphenols and Insulin Receptor Substrate (IRS) proteins, particularly IRS-1, have been discovered in recent research on the bitter melon *Momordica charantia*. Molecular docking experiments revealed these interactions, demonstrating that vanillic, ferulic, protocatechuic, p-coumaric, and ellagic acid polyphenols from bitter melon may enhance insulin sensitivity. Additionally, the results of the ADMET and bioactivity tests demonstrated that these substances adhere to Lipinski's Rule of Five, indicating their potential as therapeutic agents. The results show that chemicals from bitter melon have the potential to control insulin resistance and pave the way for more research into effective natural treatments for diabetes

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Abbreviations

ADA	American Diabetes Association
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
ATP	Adenosine Triphosphate
BME	Bitter Melon Extract
BMI	Body Mass Index
CAM	Complementary and Alternative Medicine
CB-Dock2	Community-Based Docking 2
DM	Diabetes Mellitus
DPP-4	Dipeptidyl Peptidase-4
FPG	Fasting Plasma Glucose
FRAP	Ferric Reducing Antioxidant Power
GDM	Gestational Diabetes Mellitus
GFR	Glomerular Filtration Rate
GRAVY	Grand Average of Hydropathicity
HFD	High-Fat Diet
HPLC	High-Pressure Liquid Chromatography
IRS	Insulin Receptor Substrate
KSA	Kingdom of Saudi Arabia
LPS	Lipopolysaccharide
MC	*Momordica charantia*
MODY	Maturity-Onset Diabetes of the Young
NADES	Natural Deep Eutectic Solvent
PI-3K	Phosphoinositide-3-Kinase
PKB	Protein Kinase B

PKCSM	Pharmacokinetics, Chemical Stability, Metabolism
PTNN2	Prothrombin
SSBs	Sugar-Sweetened Beverages
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TFC	Total Flavonoid Content
TPC	Total Phenolic Content

Chapter 1

Introduction

Diabetes mellitus (DM) is one of the most prevalent issues affecting public health worldwide. It is a carbohydrate metabolism condition in which insulin activity or secretion is decreased, resulting in persistently increased blood glucose levels. Diabetes is arranged into two sorts: type 1, which normally begins in childhood and is caused by immunological processes, and type 2, which usually occurs later in life, is related with aging, and is frequently caused by pancreatic diseases [1]. Prolonged hyperglycemia associated with diabetes has been linked to severe damage, abnormalities, and eventual organ failure, including the eyes, nerves, kidneys, heart, and blood vessels [2].

There is a serious threat to global health with diabetes, since 463 million adults globally suffer from this metabolic illness. Type 2 diabetes affects approximately 90% of these individuals. World Health Organization (WHO) global research on *Diabetes mellitus* was unveiled on World Health Day, April 7th, 2016, dedicated solely to addressing the challenges posed by diabetes [3]. According to a notable report by "The News," Pakistan is the third-most diabetic-prevalent country in the world, only after China and India. The data are alarming, Over time, the number of people living with diabetes in Pakistan has steadily increased. The stated prevalence rates were 11.77%, 16.98%, and 17.1% in 2016, 2018, and 2019, respectively [4].

International Diabetes Federation has raised very serious concerns over the worst situation in Pakistan. In Pakistan, the percentage of persons with diabetes increased to an astounding 26.7% by 2022. This concerning number corresponds to over 33 million instances, indicating a substantial impact on public health in the nation [3]. The significant rise in the prevalence of diabetes highlights the pressing need for better management techniques, preventative measures, and novel treatment approaches to stop the diabetes epidemic from spreading throughout Pakistan and other countries. In recent decades, both the global incidence of diabetes and The prevalence of diabetes has increased. On the other hand, although the frequency of diabetes varies throughout the world, there are regional differences in the prevalence of its complications. The frequency of diabetic complications has decreased in industrialized countries due to advancements in diagnosis, treatment, and care procedures. On the other hand, developing countries frequently struggle with inadequate diagnosis and low knowledge of appropriate management [4]. Approximately 150 million individuals globally are currently affected by diabetes, with projections indicating a doubling of this number within the next two decades [5].

One notable factor contributing to the worldwide increase in type 2 diabetes and obesity is overconsumption of calories.It is essential to keep in mind that the level of one's diet has its own independent effects on whether or not people with a higher body mass index (BMI) develop type 2 diabetes [6]. Research indicates that even after considering the effect of body weight, consuming more sugar-sweetened beverages (SSBs) builds the gamble of type 2 diabetes. According to most recent meta-analysis, individuals ate sugar-sweetened beverages (SSBs), usually averaging one or two servings daily, had a 26 percent increased chance of getting the disease as opposed to people who consumed less [7]. Insulin resistance, where the body's response to insulin decreases, along with high blood sugar levels and declining function of pancreatic beta cells, are the defining characteristics of T2DM, a major metabolic disorder [8].

The American Diabetes Association (ADA) does not recommend any specific diet for those with diabetes. It features that absolute fat utilization ought to make up

25-35% of all out calories, with immersed fat making up under 7% of all out calories and monounsaturated fat being the really fat source.. The ADA also emphasizes how important it is to consume enough fruits, vegetables, and healthy grains [9]. It also includes moderate portions of fish, poultry, dairy products such as cheese and yogurt, and occasional red wine consumption. Red meat and sugary treats are limited. Beyond its dietary components, Social and lifestyle aspects like communal dining, regular physical activity, and stress management are also included in the Mediterranean diet. Research consistently indicates that adhering to this dietary example is connected to different medical advantages, lowering the likelihood of cardiovascular disease, , type 2 diabetes, certain cancers, and cognitive decline, making it a popular and sustainable choice for promoting overall well-being [10].

Metabolic diseases are marked by a persistent state of low-grade inflammation within metabolic tissues such as the liver, adipose tissue, muscles, and pancreatic islets. Over the past decade, there has been an increased acknowledgment of the influential role of an imbalanced gut microbiota in promoting inflammatory conditions. This is achieved through its involvement in diverse signaling transduction pathways and immune responses, contributing significantly to the chronic inflammation observed in metabolic diseases . A high-fat diet (HFD) is recognized for its ability to an intestinal microbiota abundant in lipopolysaccharide, resulting in elevated LPS levels in the bloodstream. This phenomenon defines a disorder called endotoxemia [11].

Moreover,a lot of examination has been finished on the effects of alcohol use on microvascular and macro vascular problems [12]. A case study conducted in Singapore demonstrated a contrasting finding, indicating that alcohol consumption might decrease the occurrence of diabetic retinopathy. This suggests that moderate alcohol intake could potentially exert a protective influence on microvascular function [13]. Smoking represents a significant risk factor for diabetes. Smokers face an approximate 20% higher prevalence of diabetes compared to nonsmokers, with increased risk observed among individuals who commenced smoking at an earlier age and those with a longer smoking history. Additionally, older individuals are more prone to developing diabetes [14].

The pancreatic islets of Langerhans secrete the hormone insulin, which is necessary for regulating the metabolism of lipids, proteins, and carbohydrates. It does this by making it easier for the liver, skeletal muscle cells, and fat to absorb substances like glucose from the bloodstream [15].

Obesity can be made worse by insulin resistance and hyperinsulinemia. Because obesity promotes insulin resistance, it aggravates the pathophysiology of Type 2 *diabetes mellitus*. Unfortunately, our inadequate understanding of insulin resistance has hampered the management of type 2 diabetes [16]. Overstimulation of the inflammatory process can lead to a number of anomalies, such as tissue damage and organ failure. Particularly obesity has been linked to low-grade, chronic inflammation, which is crucial for the emergence of Type 2 *diabetes mellitus*. Triglyceride levels in the liver and muscles are strongly correlated with insulin resistance, especially in elderly people. Reduced ATP production and mitochondrial oxidative activity are two signs of impaired mitochondrial function that might result from these high triglyceride levels [17].

An important factor in raising insulin sensitivity is physical activity [18]. Exercise on a regular basis enhances insulin sensitivity following mechanism involves, increased glucose uptake, muscle contraction effects, improved muscle function, weight management, enhanced mitochondrial function, decreased inflammation [19]. Skeletal muscle contraction is a key mechanism in the continuous and prolonged physical activity that promotes the uptake of glucose into cells. An increase in blood flow within the muscle is a key feature of this process, which facilitates the transfer of glucose into muscle cells [20].

Diabetes can cause microvascular and macrovascular lesions by directly or indirectly triggering vascular problems through a variety of methods. Peripheral vascular problems, cardiovascular disease, and cerebrovascular disease can all be brought on by macrovascular lesions. Certain conditions such as neuropathy, retinopathy, and renal failure can be brought on by microvascular problems. Vascular problems can result from diabetes directly or indirectly through a number of different pathways. Both macrovascular and microvascular lesions are included in these consequences. In contrast to *diabetes mellitus*, the consequences resulting

from the condition are closely associated with particular body organs [21]. What's truly alarming about diabetes isn't just the disease itself, but rather the myriad complications it can lead to [22]. Self-monitoring of blood glucose (SMBG) is recognized as an essential practice for reducing the risk of diabetes complications, achieving glycemic control, and reducing the frequency of hypoglycemic episodes. Depression is a commonly identified condition with type 2 diabetes. Individuals with diabetes who also experience depression often struggle more with adhering to self-management practices compared to those without depression [24].

The body is profoundly affected by modern diets since they frequently encourage people to eat excessive amounts of carbs. Diabetes is brought on in large part by eating too many carbohydrates [23]. Understanding disparities in health and healthcare requires consideration of the concepts of "race" and "ethnicity," which play significant roles in shaping these inequities [25].

Genetic predisposition and environmental factors interact deeply to impact the development of T2DM [26]. T2DM heritability estimates range greatly, from 20% to 80%. Numerous studies, such as twin-based studies, family studies, and population-based inquiries, have produced evidence in favor of heredity [27]. Traditionally, the focus has been on genes that have already been shown to be involved in post-receptor signaling, insulin secretion, glucose metabolism, insulin receptors, and lipid metabolism [28].

Momordica charantia L., or bitter gourd in scientific parlance, is a tropical vine in the genus Momordica, order Cucurbitales, and family Cucurbitaceae Balsam pear, bitter melon, and bitter apple are all other names for it and genus Momordica. Bitter gourd is widely grown for its culinary and therapeutic properties in places like Southeast Asia, China, and India [30]. *Momordica charantia* is an annual climber plant that is monoecious. It has a thin, branching stem that can grow up to five meters tall and has clear angles and grooves on it [31].

Studies have shown that bitter gourds contains something called "plant insulin," which is a chemical that resembles insulin. It has been demonstrated that this substance is beneficial in lowering glucose levels in the blood and urine. It has

also been shown that bitter gourd contains anti-cholesterol qualities. It is well known that bitter gourd seeds are an excellent source of protein [32].

Primary metabolites of bitter gourd include common sugars, proteins, and chlorophyll. Phenolics, carotenoids, cucurbitane triterpenoids, alkaloids, and saponins are all examples of secondary metabolites. Although bitter gourd's main metabolites contribute to its basic nutritional content, its nutraceutical effects are attributed to its secondary metabolites. These substances may not add much to the bitter gourd's nutritional content overall, but they are essential for the body's production of advantageous physiological effects [33].

The bitter gourd's supposed nutraceutical benefits have been verified by numerous investigations. Among the essential characteristics are anti-diabetic effects, anti-inflammatory activity, antioxidant properties, anti-cancer potential, cardioprotective effects, immune-boosting properties, weight management, liver protection and gastrointestinal health. *Momordica charantia* is a multipurpose plant with a number of health advantages. Its abundance in vitamins, minerals, antioxidants, and other bioactive substances adds to its wide range of possible health benefits [34].

Based on research, *M. charantia*'s bioactive components have been shown to have positive effects on inflammation, scavenge free radicals, and alter cell signaling pathways. These results have been shown in investigations using animals as well as cell cultures [22]. Principal substances extracted from bitter melon and recognized as hypoglycemic agents fall into several groups. Bitter melon contains complex carbohydrates that have been demonstrated to have hypoglycemic properties. Certain proteins and peptides found in bitter melon, such as peroxidase and polypeptide-p, have been shown to have hypoglycemic qualities. Bitter melon contains compounds that have been found to have hypoglycemic properties, including charantin, which is a member of the saponins and terpenoids group. The hypoglycemic effects of quercetin, rutin, kaempferol, and isorhamnetin, among other phenolic compounds, have been linked to bitter melon. Polypeptide-p is a well-known substance that was taken from the bitter melon plant for medicinal purposes [35].

The seeds and fruit of *M. charantia* include important functional components that are proteins and peptides. Peroxidase is one of the a number of additional polypeptides that have been found in the parts of plants [36].

Isolated phytoconstituents from native herbs offer a viable avenue for the creation of new medicines. As such, a great deal of study is being done on traditional herbs. Among these herbs is *Momordica charantia* (MC), a member of the Cucurbitaceae family, which has been used for millennia in medicine and cooking. It is especially valued for its ability to prevent diabetes [37].

Momordica charantia exhibits a pharmacological profile that is exceptionally varied [38]. It has demonstrated several positive effects, such as: Blood sugar regulation is one of its well-known antidiabetic qualities. Effective against a range of bacterial illnesses, antibacterial action [39]. Antiviral effects Demonstrates efficacy against viruses, including poliovirus, herpes, and HIV. Anticancer properties Shows promise in preventing the proliferation of cancer cells. Effects of antifertility may have an impact on reproduction and fertility [40]. Anti-ulcer activity aids in preventing the development of ulcers.Impacts of immunomodulation adjusts the immune system. Benefits those who suffer from psoriasis due to its antipsoriatic characteristics [41]. Pain and inflammation are reduced by the analgesic and anti-inflammatory actions. Hypotensive activity: Shows signs of reducing blood pressure. Properties of antiprothrombin: May affect blood coagulation. Effects of hypocholesterolemia: Lowers cholesterol levels. Antioxidant activity: Removes damaging free radicals from the body [42].

Momordica charantia leaf extract has shown antibacterial efficacy against a variety of pathogens, such as Salmonella paratyphi, Escherichia coli, Shigella dysenteriae, and Streptomyces griseus [43]. Diabetes is caused by a number of pathological processes, including dysfunctions that result in insulin resistance and the autoimmune death of pancreatic beta cells, which results in insulin deficit. Noticeable hyperglycemia symptoms include polyuria, polydipsia, weight loss (often combined with poly phagia), and impaired vision [38].

A qualitative phytochemical examination showed that the studied plants contain alkaloids, flavonoids, saponins, phenols, and tannins. The quantitative analysis indicated that bitter leaf possesses the highest levels of alkaloids and flavonoids among the plants examined. Polyphenols are caffeic acid, rutin, kaemferol, isoquercetin, myricetin, syringic acid catechins, epicatechins, luteolin, gallic acid, vanillic acid, ferulic acid, p-coumaric acid, ellagoc acid [35].

To better comprehend the functions of proteins, anticipate molecular interactions, and identify and study novel therapeutic molecules, diabetes researchers employ a variety of online tools and methods. The Protein Data Bank provided us with the IRS (Insulin Receptor Substrate) main sequence in FASTA format. This grouping is essential for additional investigation and demonstrating [80].

ProtParam, a method for predicting theoretical pI, molecular weight, number of amino acids, and positively and negatively charged residues, was used to investigate the IRS protein's physicochemical properties. Understanding these characteristics aids in determining the stability and function of the protein. Using information from the PDB, a 3D structure for the IRS protein was predicted. This data set contains an abundance of primary data essential for layout based demonstrating, approval, and refinement, which are all expected for dependable protein structure expectation [81].

ProtParam is used to investigate the IRS protein's physicochemical properties. This program predicts different characteristics, including hypothetical pI, subatomic weight, measure of amino acids, and decidedly and adversely charged buildups. The protein's activity and stability, which are essential for its participation in insulin signaling pathways, can be defined by gaining an understanding of these properties [82].

The Protein Data Bank (PDB) has a lot of structural information that can be used to figure out the IRS protein's three-dimensional structure. The protein structure can be modelled, validated, and refined using this data, which improves our comprehension of its interactions with potential plant-based medicinal chemicals.

Protein structures can be analyzed and visualized in three dimensions using PyMOL. This atomic designs device permits you to control particles, produce beam followed pictures, and make activitys. For gaining an understanding of how plant-derived chemicals interact with the IRS protein on a molecular level, visualizing its structure is extremely helpful [83].

1.1 Problem Statement

Current synthetic diabetes medications provide symptomatic relief with side effects. Investigating bioactive compounds from okra leaves could offer natural, holistic solutions for managing and treating Type 2 diabetes.

1.2 Hypothesis

In silico analysis will reveal that polyphenols of *Momordica charantia* leaves will be helpful to mitigate the type 2 diabetes.

1.3 Aim

This study aims to computationally investigate the interactions between bioactive compounds in *Momordica charantia* leaves and the IRS gene, to understand how these interactions may enhance insulin signaling pathways in diabetes.

1.4 Objectives

1. Identify key polyphenols in *Momordica charantia* that may interact with the IRS gene.
2. Analyze the binding conformation between the IRS gene and polyphenols as standard antidiabetic agents.

3. Identify a lead compound as a potential antidiabetic drug candidate.

Chapter 2

Review of Literature

2.1 Type 2 *Diabetes Mellitus* (T2DM)

T2DM is characterized by the dysregulation of protein, lipid, and carbohydrate metabolism, stemming from factors such as insulin resistance, insufficient insulin secretion, or a blend of both [44]. Among the various types of diabetes, T2DM stands out as the most common, accounting for over 90% of diagnosed cases, surpassing the widespread use of both type 1 *Diabetes Mellitus* (T1DM) and gestational diabetes [45]. Diabetes encompasses a group of chronic disorders categorized into four main types: Type 2 *Diabetes Mellitus* (T2DM), gestational *Diabetes Mellitus* (GDM), and monogenic diabetes are all forms of diabetes. Dysregulated glucose metabolism is the result of autoimmune destruction of pancreatic beta cells in type 1 diabetes. In contrast, beta cell dysfunction and systemic insulin resistance are the causes of type 2 diabetes. Insufficient insulin production or glucose intolerance during pregnancy lead to gestational diabetes (GDM). Ineffectively oversaw GDM can prompt sort 2 *Diabetes Mellitus* (T2DM), a constant condition. A less common type of diabetes caused by genetic mutations is monogenic diabetes, which is also known as maturity-onset diabetes of the young (MODY) changes in a single characteristic. It can sometimes be mistaken for T1DM or T2DM [46].

2.2 Traditional and Herbal Remedies in Diabetes Management

Complementary and alternative medicine (CAM) encompasses various non-conventional medical systems, medications, supplements, and treatments, including herbal medicine. Herbal medicine, a component of CAM, utilizes plant-derived substances for disease prevention and treatment. Despite its ancient roots spanning 5000 years, there remains limited scientific evidence supporting the efficacy of herbal medicine. Nevertheless, approximately 80% of individuals in numerous countries, particularly in developing regions like Asia and Africa, regularly rely on herbal remedies for primary healthcare [47].

In developed countries such as France and the US, something notable rise in demand for herbal medicine, with 75% and 42% of the population, respectively, having used herbal remedies at least once in the past two decades. In KSA, herbal medicine holds a significant position within complementary and alternative medicine (CAM) practices, extensively utilized for medicinal purposes by the general population. Despite the widespread use of herbal remedies, particularly in managing chronic conditions such as diabetes, there is a lack of research on the attitudes and perspectives of healthcare professionals and patients regarding their use [47].

2.3 Bitter Gourd (*Momordica charantia*)

Momordica charantia is a widely recognized plant utilized by numerous indigenous populations worldwide for managing diabetes-related conditions. Its name derives from the distinctly bitter taste of its fruit, particularly when ripe. While biochemical and animal model studies have generated significant data and hypotheses regarding the potential anti-diabetic properties of *Momordica charantia*, clinical trials involving human subjects remain limited and often suffer from poor design quality [47].

2.4 Plant Description

Momordica charantia, a member of the Cucurbitaceae family, is also known as bittermelon or bitter gourd. This blooming vine is widely planted in tropical locations including Asia, India, East Africa, and South America. Its fruits are renowned for their intensely bitter taste and are frequently employed in culinary practices and as a natural remedy for diabetes. Typically growing as a climbing perennial reaching heights of up to 5 meters, *Momordica charantia* produces elongated fruits with a uneven surface. Due to its significant nutritional and therapeutic properties, *Momordica charantia* stands out as one of the most promising herbal remedies for diabetes management [48].

2.5 Phytochemistry

The anti-diabetic effects of bitter melon are linked to a broad array of chemical constituents. These include triterpenes, proteins, steroids, alkaloids, lipids, phenolic compounds, glycosides, saponins, resins, fixed oils, free acids, and various other bioactive substances. Research indicates that *Momordica charantia* is rich in nutrients, with its leaves and pulp serving as excellent sources of calcium, magnesium, potassium, phosphorus, iron, and B vitamins [48].

Momordica charantia is a nutrient-rich plant packed with essential nutrients and bioactive compounds. Its diverse array of beneficial components, including vitamins, minerals, antioxidants, and bioactive substances, contribute to its therapeutic potential and medicinal properties [49].

2.6 Chemical Composition of Bitter Gourd Leaves Extract

Foods containing chemical substances have the capacity to regulate metabolic functions in both animals and humans. These bioactive ingredients are mostly

present in fruits, vegetables, and whole grains. Possess the capability to restore insulin release, regenerate pancreatic β cells, and reverse insulin resistance and prevent metabolic diseases [50].

A phytochemical analysis of two hybrid variants of M. charantia revealed the existence of alkaloids, flavonoids, tannins, and saponins in extracts obtained using Ethyl acetate and petroleum ether for both GOJNEE and TIA varieties.

However, alkaloids were not detected in the petroleum ether extract for either variety. This suggests that both varieties of M. charantia extracts contained nearly identical phytochemical constituents, with comparable percentages of alkaloids, flavonoids, and saponins [51].

2.6.1 Anti-Diabetic Activity

An illness of the metabolism is *Diabetes Mellitus* marked by high levels of sugar in the blood due to deficiencies in insulin secretion, insulin activity, or both.

Momordica charantia, a traditional treatment employed for centuries in alternative and complementary medicine, has been utilized for managing diabetes [52].

2.6.2 Anticancer Property

Over the past few decades, numerous initial trials have been undertaken to uncover and confirm *Momordica charantia*'s anti-cancer potential. Research indicates that bioactive compounds found in *M. charantia* play a role in regulating various types of cancer including cervical, breast, liver, nasopharyngeal, leukemia, and colon cancers.

Regardless of a few in vitro and in vivo examinations investigating its efficacy against carcinoma, orderly clinical preliminaries involving cancer patients are necessary to validate the anti-cancer properties of *M. charantia* [53].

2.6.3 Anti-oxidative Property

Research investigate the impact of *Momordica charantia* and its specific compounds on oxidative stress, a primary factor in the development of lifestyle-related diseases like hypertension, diabetes, and obesity. Many studies suggest that bitter gourd exhibits significant antioxidant properties, demonstrating superior antioxidant capacity compared to colocasia.

A study conducted in mice, where they were administered suitable doses of bitter gourd polysaccharide, revealed its ability to neutralize peroxide free radicals generated in the body, inhibit the chain reaction of free radicals, and contribute to antioxidant and anti-aging mechanisms [52].

2.6.4 Anti-dementia Activity

Neurodegenerative disorders disrupt brain cell communication, resulting in an irreversible impairments in speech, movement, and memory, and cognitive abilities. These conditions involve progressive degeneration of specific neurons due to metabolic or toxic stresses. In a study by Tamilanban (2018), charantin was removed from *M. charantia* and its neuroprotective effects were assessed through using neuroblastoma cell lines from SH-SY5Y for in vitro research. A mixture of sigmasterol glucoside and -sitosterol glucoside makes up charantin, a steroidal glycoside [53].

2.6.5 Antibacterial Activity

The most effective concentration of bitter melon leaf extract in inhibiting A. hydrophila was found to be the P3 treatment, with a concentration of 5 mg/ml, resulting in the largest inhibitory zone on average. This antibacterial effect was attributed collective a variety of phytochemical compounds found in the bitter melon leaves, including alkaloids, flavonoids, tannins, and saponins. The mechanism of action of alkaloids involves inhibiting nucleic acid synthesis and also

blocking the dihydrofolate reductase enzyme, which contributes to the inhibition of A. hydrophila growth [54].

2.6.6 Medicinal Uses

Bitter melon is renowned for its therapeutic properties, encompassing cholesterol reduction, diabetes management, cancer prevention, and inflammation reduction. It contains numerous phenolic compounds with potential antimutagenic and antioxidant properties.Different parts of the bitter melon plant, including its fruit, stems, leaves, and roots, have been used in traditional medicine to treat a wide range of conditions, including menstrual irregularities, microbial infections, digestive disorders, and hyperlipidemia. Its potent antiviral properties contribute to combating infections like human immunodeficiency virus (HIV) and white spot syndrome virus by enhancing natural killer cells and bolstering the immune system [48].

Studies indicate that *Momordica charantia* exhibits anti-carcinogenic properties and serves as a cytotoxic agent against various types of cancer. In line with research conducted by Ray et al., bitter melon extract shows promise as a dietary supplement for breast cancer prevention. It achieves this by regulating signal transduction pathways, hindering the development of bosom malignant growth cells [48].

2.6.7 Previous Studies on *Momordica charantia* and Diabetes Management

Research was conducted in 2005 and checked the effect of *Momordica charantia* on Streptozotocin-induced diabetic rats' glucose levels. In this study, male Wistar rats were induced with Type I diabetes using streptozotocin. Those in the starch-fed diabetic group (SFD) showed typical diabetic symptoms, including increased water intake, polyphagia, and polyuria with high urine sugar excretion. However, incorporating bitter gourd into the diet (BFD) significantly reduced water intake,

urine excretion, and urine sugar excretion. Bitter gourd also partially prevented renal hypertrophy and significantly reduced the glomerular filtration rate (GFR) in diabetic rats. These findings suggest bitter gourd's potential in managing diabetic symptoms and protecting against kidney complications. Further research is needed to understand its mechanisms in diabetes management [55].

In 2014 another research were conducted against diabetic patients. The effect of Mormordica charantia was checked on diabetic patients. Mormordica charantia (bitter melon) has been studied for its ability to lower plasma glucose in diabetics. The final result was that three trials, encompassing a total of 187 participants, met all criteria for inclusion.

However, only two trials provided useful information for A1c meta-analyses, while two trials reported data for FPG. All selected studies focused solely on individuals with type 2 diabetes, despite the possibility of including type 1 diabetes patients in the search strategy [56].

Study was conducted in 2022 aimed to determine the taste acceptability and healing effect of the bitter gourd by formulating a drink for diabetic patients.The study evaluated five bitter gourd drink formulations among diabetic participants, finding that Formulation 5, containing multiple fruit extracts alongside bitter gourd, received the highest acceptability ratings.

It emphasized the role of fruit sweeteners in enhancing taste and effectiveness, recommending their use alongside bitter gourd extract. Additionally, exploring less bitter varieties like Indian Karela was suggested for improved palatability [57].

Study was conducted in 2023. It was a 2-week study with prediabetic patients, bitter melon extract (BME) was found to reduce blood glucose levels, particularly 30 minutes after oral glucose intake. Additionally, BME led to a significant decrease in glucagon levels after 120 minutes of glucose ingestion, suggesting its potential in lowering glucose levels by suppressing glucagon in individuals with prediabetes [58].

2.7 IRS Proteins

IRS proteins transmit insulin and IGF-1 receptor signals to elicit cellular responses as cytoplasmic adaptor molecules. It was initially discovered that IRS-1, the first member of this family, was a 185 kD phosphoprotein that reacted with insulin stimulation. As a result, insulin-invigorated cells from Irs-1-/- mice contained IRS-2, previously known as 4PS, an elective insulin receptor substrate. The essential middle people of insulin-subordinate mitogenesis and the guideline of glucose digestion in an assortment of cell types, IRS-1 and IRS-2 are generally dispersed [59].

Although IRS-4 is also present in humans, its expression is more restricted, particularly in the brain, thymus, and liver. The symptoms observed in knockout mice demonstrate, despite their striking similarity, that IRS proteins carry out distinct and crucial functions. Irs-1-/ - mice are brought into the world around 70% more modest than wild-type mice and hold this size all through their lives, showing that this IRS protein assumes a significant part in controlling organismal development. Irs-2-/- mice, on the other hand, have abnormalities specific to specific tissues but maintain normal body size [59]. Specifically, these mice have diminutive brains attributed to a 50% decrease in neuronal growth, reduced photoreceptor cells, and female infertility stemming from small, anovulatory ovaries along with pituitary abnormalities [60].

2.8 *Momordica charantia*: Bioactivities Associated with Diabetes, Quality Assurance, and Safety Issues

In 2022, a study explored *Momordica charantia*, initially focusing on charantin, a blend of two sterol glucosides, and the polypeptide p-insulin [61]. However, their low concentrations in the fruit and limited bioavailability did not explain the

reported therapeutic effects [62]. Consequently, further investigation into potentially active components was halted. Triterpenoids of the type cucurbitane, which are prevalent throughout the plant, have been the focus of recent research. This review examines bitter gourd-derived compounds, assessing their bioactivities for potential antidiabetic effects or adverse reactions. It also evaluates methodologies for assessing the quality of bitter gourd fruits and preparations, aiming to establish their relevance and potential for standardizing commercial products [63].

The isolates demonstrated inhibitory activity ranging from 72 to 93%. While all isolates were evaluated for their ability to inhibit α-glucosidase, none exhibited a significant effect compared to acarbose [63].

2.9 Polyphenol

Bitter gourd leaves contain a diverse array of polyphenolic compounds, including catechins, which belong to a chemical family known for their antioxidant properties [64]. Polyphenols found in bitter gourd leaves, as well as other plants, have been studied to assess their potential impact on diabetes and related conditions. There is an overview of various polyphenols commonly found in bitter gourd leaves, indicating those that have been reviewd for their effects on diabetes and those that have received less specific attention in the realm of diabetes research [64].

Catechins, Gallic acid, Chlorogenic acid, Epicatechins, Caffeic acid, Quercetin. Luteolin, Kaempferol, Rutin, Apigenin, Myricetin, yringic acid these polyphenols have reviewd on diabetes. While Vanillic acid, Ferulic acid, Protocatechuic acid, P-coumaric acid, Ellagic acid shows less research on diabetes. Polyphenols like catechins, quercetin, and chlorogenic acid have been extensively studied for their possible advantages in combating diabetes [64].

IRS (Insulin Receptor Substrate) proteins are like messengers inside our cells. They carry signals from insulin and similar hormones to pathways that control things like 1how our body uses energy, grows, and stays alive [65]. Metformin is a commonly prescribed oral drug that increases insulin sensitivity and reduces

glucose synthesis in the liver [66]. Sulfonylureas stimulate the pancreas to release more insulin, which helps to lower blood sugar level Examples include glyburide, glipizide, and glimepiride [67]. DPP-4 Inhibitors work by blocking the enzyme dipeptidyl peptidase-4 (DPP-4), which controls blood sugar levels by increasing insulin release and decreasing glucagon production. Common examples include sitagliptin, saxagliptin, and linagliptin [68].

2.10 Role of Insulin Receptor Substrate Proteins in Cellular Signaling and Integration

The discovery of insulin receptor substrate (IRS) proteins and their job in associating cell surface receptors to intracellular flagging pathways is pivotal for grasping the activities of insulin and insulin-like development factors (IGF). Additionally, IRS proteins integrate signals from insulin and IGF receptor tyrosine kinases with those generated by proinflammatory cytokines and nutrients [69]. Insulin signal transduction is dependent on IRS proteins, specifically IRS-1 and IRS-2. When insulin interacts to its receptor, IRS proteins are tyrosine phosphorylated, which activates signaling cascades such as the PI3K/Akt pathway. As a result, several cellular responses are activated, including glucose absorption, glycogen synthesis, and lipid metabolism. Dysregulation of IRS protein function is an indication of insulin resistance and T2DM [70].

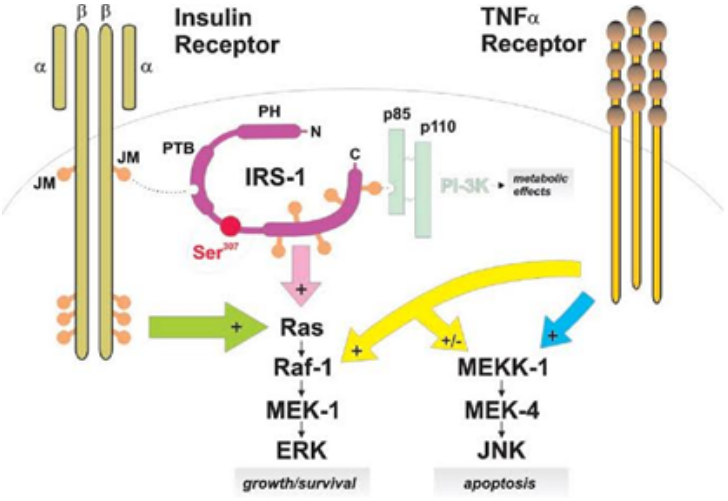


FIGURE 2.1: Mechanism of action of IRS

2.11 Mechanism of Action of IRS

When insulin binds to its receptor, a transmembrane protein found on the outer layer of insulin-responsive cells (such as muscle, liver, and fat tissues), it causes a conformational change in the receptor. This change Activates the intrinsic tyrosine kinase activity of the receptor, resulting in the autophosphorylation of certain tyrosine residues in the intracellular region [71].

The insulin receptor is a transmembrane glycoprotein that belongs to the receptor tyrosine kinase (RTK) family. The heterotetrameric structure ($\alpha 2 \beta 2$) is made up of two extracellular alpha and two transmembrane beta subunits [72].

Alpha subunits: entirely extracellular and contain the insulin-binding site.

Beta subunits: bridge the cell membrane and have intrinsic tyrosine kinase activity in their cytoplasmic regions.

When insulin is released into the bloodstream when blood glucose levels are too high, It gets to the tissues it wants. and attaches to the extracellular alpha subunits of insulin receptors. Binding of insulin to its receptor causes a conformational change in the structure of the receptor, which is required to activate the receptor's tyrosine kinase function. Insulin interaction causes a conformational shift that puts the beta subunits' intracellular kinase domains into close contact, resulting in autophosphorylation of certain tyrosine residues on the receptor's cytoplasmic tail [73].

Autophosphorylation has several applications. First, it increases the receptor's kinase activity, which allows it to phosphorylate other proteins. Second, the phosphorylated tyrosine deposits act as docking destinations for intracellular signaling proteins with SH2 (Src homology 2) domains, which recognize and bind to the phosphorylated motifs [74].

The insulin receptor substrate (IRS) proteins, especially IRS-1 and IRS-2, are key substrates for the active insulin receptor. IRS proteins are attracted to the phosphorylated insulin receptor via the PH (pleckstrin homology) and PTB

(phosphotyrosine-binding) domains, which connect with specific phosphotyrosine residues on the receptor. The insulin receptor phosphorylates IRS proteins at several tyrosine residues, resulting in more docking sites for downstream signaling molecules [75].

2.12 Activation of Downstream Signaling Pathways

Phosphorylated IRS proteins function as scaffolding, attracting and activating a variety of downstream signaling molecules and pathways. PI3K is a crucial signaling molecule that interacts to phosphorylated IRS proteins [76].

2.12.1 PI3K/Akt Pathway

PIP3 is made when PI3K is activated. (phosphatidylinositol-3,4,5-trisphosphate), which attracts and activates Akt. Akt mediates insulin's metabolic actions, including as glucose absorption, glycogen synthesis, and lipid metabolism.

2.12.2 MAPK Pathway

The MAPK (mitogen-actuated protein kinase) pathway is additionally enacted by IRS proteins, resulting in gene expression alterations that promote cell proliferation and differentiation [77].

2.13 Cellular Responses to Insulin

The activation of these downstream pathways triggers a variety of cellular responses that are required to maintain glucose homeostasis.

2.13.1 Glucose Uptake

The translocation of GLUT4 is aided by Akt (glucose transporter type 4) to the cell membrane by vesicles, hence enhancing glucose absorption in muscle and adipose tissue [78].

2.13.2 Glycogen Synthesis

Akt inhibits GSK3 (glycogen synthase kinase 3), increasing the activity of glycogen synthase and promoting glycogen storage in the liver and muscles [78].

2.13.3 Lipid Metabolism

Insulin signaling modulates enzymes involved in lipogenesis (fat production) and lipolysis (fat breakdown), hence controlling lipid metabolism.

There are various regulatory mechanisms in place to guarantee precise control over insulin signaling. Protein tyrosine phosphatases (PTPs) can dephosphorylate the insulin receptor and IRS proteins, thereby stopping the signal. Furthermore, serine/threonine phosphorylation of IRS proteins by various kinases can impair their activity and avoid excessive insulin signaling [79].

2.14 Insilico Tools Used to Check the Senstivity of IRS1

Chronic hyperglycemia is a hallmark of diabetes mellitus, a common metabolic disorder brought on by malfunctions in insulin secretion, insulin action, or both. Plant extricates have for quite some time been examined for their capacity to change insulin pathways and proposition elective medicines. The study of these

plant-derived substances has been transformed by the combination of bioinformatics and computational methods, allowing for extensive research and validation of their medicinal properties [80].

2.14.1 Retrieval of Sequence

The fundamental grouping of target proteins, like the Insulin Receptor Substrate (IRS), is basic for understanding how they work and communicate with remedial medications. These sequences are saved in the Protein Data Bank (PDB). Past examination have downloaded the IRS grouping in FASTA design from the PDB to help future examinations and demonstrating. Smith utilized the IRS arrangement got from PDB in 2020 to look at its primary and useful highlights, which supported the recognizable proof of conceivable association locales for plant-based drugs [80].

2.14.2 ProtParam

Protein dependability and capability are exceptionally reliant upon their physicochemical characteristics. ProtParam is an internet based device that predicts boundaries including hypothetical pI, sub-atomic weight, and how much charged buildups. The IRS protein was studied using ProtParam by Johnson et al. (2019), which revealed its activity and potential interactions with chemicals derived from plants [82].

2.14.3 PDB

Knowledge of proteins' three-dimensional structure is necessary to comprehend how they interact with potential medical treatments. Template-based modeling and validation can benefit from the extensive structural data provided by the PDB. The accuracy of molecular docking studies was improved as a result of the use of this data to estimate the IRS protein's three-dimensional structure [83].

2.14.4 PyMOL

PyMOL, a sub-atomic designs device, is ordinarily used to picture and dissect protein structures. It makes it possible for scientists to control molecules, make detailed photographs, and make animations. Utilizing PyMOL, Thompson demonstrated the interactions between IRS proteins and ligands derived from plants, assisting in the development of more potent therapeutics [87].

2.14.5 InterPro

InterPro is used to find the functional domains of the IRS protein. This web-based data set centers around monitored spaces associated with grouping and primary cooperations, uncovering utilitarian components of the protein that are basic to its job in insulin flagging and glucose digestion. researcher used this tool to refine the chemical structures of ligands, such as polyphenols and other active chemicals found in Momordica charantia plant extracts. These ligands were chosen for their ability to alter IRS protein activity and their pharmacological properties [94].

2.14.6 PkCSM

To guarantee an accurate structural representation, canonical smiles are retrieved from PubChem when necessary, loaded into ChemDraw, and then modified using ChemPro software. PkCSM is used to evaluate these plant-derived substances' ADME/T characteristics [89].

2.14.7 CB-Dock2

This apparatus approves that the chose compounds comply with Lipinski's Standard of Five, anticipating their pharmacokinetic highlights and conceivable restorative adequacy. For the purpose of determining the safety and effectiveness of plant extracts in the treatment of diabetes, it is essential to comprehend these characteristics. CB-Dock2 is utilized to perform sub-atomic docking, a computational

methodology that predicts the limiting system and fondness of little particle ligands to protein targets. By automating the prediction and docking of binding sites using this online docking program, researchers can examine how plant-derived chemicals interact with the IRS protein. The creation of files for both the ligands and the target protein is the first step in molecular docking [91].

Chapter 3

Research Methodology

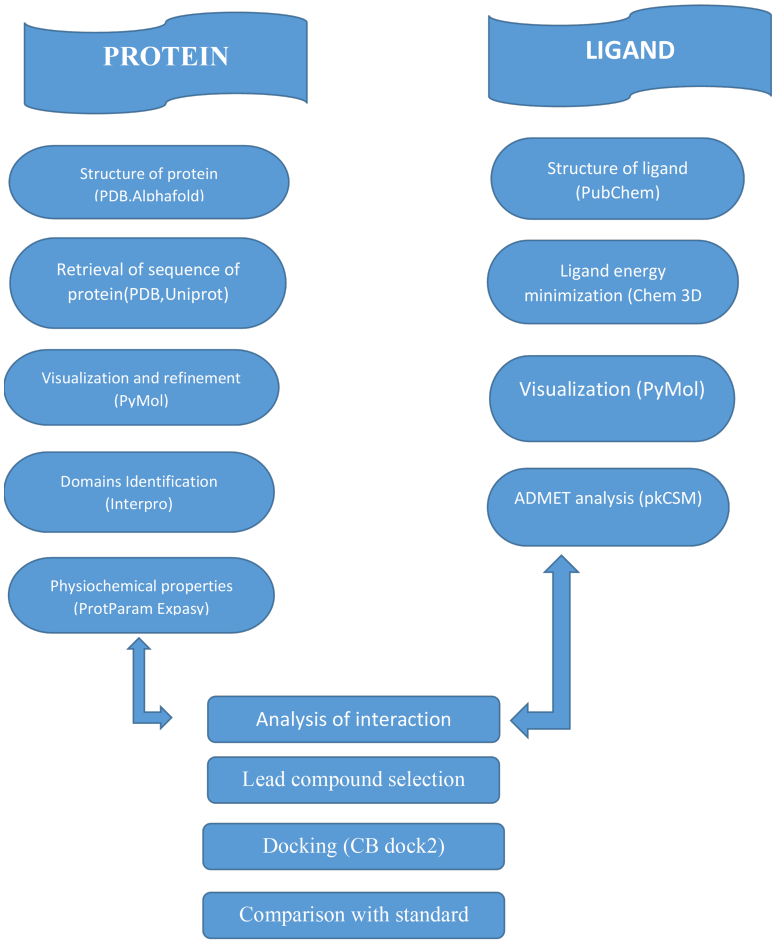


FIGURE 3.1: Methodology Flowchart

3.1 Problem Identification and Selection

The rising incidence of the role that insulin resistance plays in metabolic disorders like type 2 diabetes necessitates novel therapeutic approaches. Momordica charantia (bitter melon) polyphenols such as vanillic acid, ferulic acid, protocatechuic acid, P-coumaric acid, Ellagic acid have been suggested to enhance insulin sensitivity, but the molecular interactions with the Insulin Receptor Substrate (IRS) protein remain unclear.

This in silico study investigates the effects of Momordica charantia leaf polyphenols on the sensitivity of IRS protein, using computational modeling and molecular docking techniques to elucidate potential mechanisms and interactions. Understanding these interactions may lead to the development of new therapeutic agents targeting insulin resistance.

3.2 Target Protein Selection

In the in silico study on the impact of Momordica charantia leaf polyphenols on Insulin Receptor Substrate (IRS) proteins, IRS-1 is chosen as the target protein because of its essential role in insulin signaling and its significant association with insulin resistance.

The goal will be to obtain a high-quality, high-resolution structure of IRS-1, including its critical functional domains.

3.3 Retrieval of the Primary Sequence

Primary target protein sequence (IRS) was taken in FASTA format from protein data bank [80].

3.4 Analyses of Protien's Physical and Chemical Properties

Physicochemical properties play a crucial part in determining the purpose of proteins. ProtParam web.expasy.org/protparam was used to predict these properties of IRS protien. Theoretical pI, molecular weight, number of amino acids, number of positively charged residues, and number of negatively charged residues (Asp + Glu) [81].

3.5 3D Structure Prediction of Protein

The 3D structures can be predicted through PDB www.rcsb.org. The Protein Data Bank is an essential resource for 3D protein structure prediction. It supplies foundational data crucial for template-based modeling, validation, refinement, and benchmarking of predictive algorithms. Utilizing the extensive structural information in the PDB allows researchers to improve the accuracy and reliability of protein structure predictions, thereby advancing drug design, functional annotation, and biotechnology [80].

3.6 Structure Analysis by Use of PyMOL

PyMOL pymol.org/ is a strong cross-stage sub-atomic illustrations application utilized worldwide for three-layered investigation and representation of proteins, small molecules, nucleic acids, electron densities, surfaces, and trajectories. Its powers include manipulating molecules, generating ray tracing, and creating animations and movies. PyMOL, which is built on Python, includes several plugin tools that extend its capability, making it an invaluable resource for drug targeting and design. Its interaction with other software, such as MIX, expands its utility

and applications in molecular biology and drug development. After downloading the protein structure, additional components connected to the protein were eliminated using the open-source platform PyMOL [82].

3.7 Functional Domain Identifification of Target Protien

Interpro www.interpro.com is an online database which was used to identify the functional domains of targeted protein IRS. Conserved domains are involved in sequence/structure/relationship [83].

3.8 Retrieval of Chemical Structure of Ligands

PubChem pubchem.ncbi.nlm.nih.gov is the biggest world's archive of easily available chemical knowledge. Ligands utilized in this investigation were chosen from the PubChem database and refined using ChemDraw Ultra version 12.0.2 software. The following ligands were chosen for their pharmacological properties: Vanillic acid, Ferulic acid, Protocatechuic acid, P-coumaric acid, Ellagic acid. In cases where the desired ligand structures were unavailable, we obtained canonical smiles from PubChem, imported them into ChemDraw, and repeated the energy minimization phase with ChemPro software for additional refinement [84].

3.9 Ligand Bioactivity Studies and Toxicity Measurement

Compounds that were utilized as ligand were selected from PubChem database. Selected compounds follow the lipinski rule of fifive and those are likely to be used as active drug in humans. The potential success of a compound depends on its ADME/T properties. PkCSM omictools.com/pkcsm-tool is an online tool that

helps to fifind the ADME/T properties of the compounds. The rules are described as under:

1. The logP value of molecules should be limited to 5.
2. Maximum number of H-bond acceptor should be 10.
3. Maximum number of H-bond donor should be 5.
4. Molecular weight (MW) should be less than 500 daltons [85].

3.10 Molecular Docking of Targeted Proteins

Molecular docking refers to the computational technique used to predict the binding mode and affinity of a small molecule ligand (such as a drug candidate) to a protein target. This process involves simulating the interaction between the ligand selected through literature review and the protein's binding site to determine how they interact and form a stable complex [86].

CB dock2 clab.labshare.cn/cbdock/blinddock is an online docking service that automatically finds binding sites and performs docking operations. Its feature speeds up the docking process and improves precision by predicting the binding locations of target proteins [86].

3.10.1 Process of Molecular Docking

The initial phase of the docking process is creating files for both the ligand and the target protein. First, the target protein file is built in multiple steps. Each target protein's PDB file is successively loaded into CB-Dock. The target protein file is then saved in pdbqt format once any necessary alterations have been performed. Following the compilation of protein files, a similar technique is used to prepare ligand files, which are saved in PDB format in the same directory [87].

Next, a grid box is created around the protein-ligand structure. This entails choosing the macromolecules option from the Grid menu and opening the pdbqt target protein file. The ligand structure is then opened using the set map type option. Once these procedures are accomplished, the grid box option is used to create a grid around the protein-ligand complex. Adjust the grid box parameters, including all essential values, and save the file as a Grid Parameters File in the same directory [87].

Docking-specific commands are used to start the process. These commands help to identify the directory path where the produced protein-ligand files, as well as the grid parameters file, are saved. This stage generates docking files for the given dataset and saves the results in pdbqt format [88].

3.10.2 Active Site Identifification

The ligand exhibits the strongest interaction with the protein at the target protein's active site. Amino acids play a crucial role in the formation of the ligand-protein complex. Protein binding pockets were identified using CASTp, software sts.bioe.uic.edu/castp which provides detailed insights into the topography and area of these pockets. This identification is essential for understanding the specific regions where the ligand is likely to bind, ensuring accurate docking and interaction analysis [89].

3.11 Protein Ligand Interaction

The interactions between the active pockets of the ligand and the protein are calculated to interpret the docking results. Two types of interactions are analyzed: hydrogen bonding and hydrophobic interactions. Protein-ligand interactions were studied using LigPlot+ (version 1.4.5), a software that automatically generates schematic diagrams of these interactions based on the provided PDB files. This visual representation helps in understanding the nature and strength of the interactions between the ligand and the protein [90].

3.12 Lead Compound Identification

In the wake of directing top to bottom examination of protein-ligand collaborations, docking scores, and toxicity studies, the most potent inhibitor was identified. Protocatechuic Acid was chosen as our lead compound.

3.13 Comparison with Reference Drug

The most efficient inhibitor was discovered through extensive docking scores, toxicity studies, and interaction studies between protein and ligand

Chapter 4

Results and Discussions

This chapter summarizes the findings gained through our methodological processes. The 3D structures of the proteins and ligands served as inputs. After analyzing physicochemical parameters and predicting domains, the proteins were docked with the chosen ligands, whose energies had previously been reduced. The compounds' drug-like qualities were predicted using ADME/T attributes and Lipinski's rule. The identified chemical was further validated by comparing its properties to those of an existing antibiotic medication. The following headings provide detailed descriptions of each of these steps.

4.1 Structure Modeling

Structure modeling includes primary sequence retrieval, physiochemical properties prediction, 3D structure prediction and functional domain identification of proteins.

4.1.1 Retrieval of Sequence

Retrieving the primary sequence of a protein is a crucial step. This process typically involves accessing reliable databases such as UniProt or the Protein Data Bank (PDB) and searching for the protein of interest using specific identifiers or

keywords. The sequence of IRS1 was retrieved by using PDB https://www.rcsb.org/. This protien were selected on the basis of central role in insulin signaling pathways, impacting glucose metabolism and cellular growth [91]

```
>1IRS_1|Chain A|IRS-1|Homo sapiens (9606)
MGPAFKEVWQVILKPKGLGQTKNLIGIYRLCLTSKTISFVKLNSEAAAVVLQLMNIRRCGHSENFFFI
EVGRSAVTGPGEFWMQVDDSVVAQNMHETILEAMRAMSDEFRPR
```

FIGURE 4.1: Sequence Retrieval

4.1.2 Physiochemical Characterization of Proteins

Proteins are physiochemically characterized by looking at factors like molecular weight, isoelectric point (pI), amino acid content, and instability index. Protparam, an online tool given by the ExPASy Bioinformatics Resource Portal, is essential to this process. By entering a protein's amino acid sequence into Protparam, i got a thorough examination of its physiochemical properties. This includes computing characteristics such molecular weight, theoretical pI, amino acid content, and GRAVY (grand average hydropathicity).

The calculated PI greater than 7 represents the basic nature of the protein while less than 7 shows acidic nature of protein. Extinction coefficient represents light absorption. Instability index if less than 40 show stability of the protein while greater than 40 indicates the instability of protein.These metrics provide important insights into proteins' structural and functional properties, which aids in their characterization and understanding of their functions in biological processes [92].

TABLE 4.1: Physicochemical properties of target proteins

Protein	**Poperties**	**Score**
	Number of amino acid	112
	Molecular weight	12633.75
	Theoretical pI	8.71
	-ive residues	11
IRS1	+ive residues	13
	Total atom	1782

Table 4.1 continued from previous page

Protein	Poperties	Score
	Instability index	38.8
	Aliphatic index	88.75
	Grand average of hydropathicity	0.013

The aliphatic index measures a protein's aliphatic composition. A high aliphatic index shows the protein's thermal stability. Protein residues can be both positively and negatively charged, as shown by their molecular weight. Low GRAVY indicates better interactions with water molecules [92].

4.1.3 Predicting the 3D Structures of Proteins

3D structure prediction of the IRS1 protein using the Protein Data Bank (PDB). The primary objective was to elucidate the structural characteristics of IRS1 to better understand its role in insulin signaling pathways and metabolic disorders. By utilizing available structural data from the PDB, including homology modeling and molecular docking techniques, generated a predicted 3D model of the IRS1 protein. This model provided insights into the spatial arrangement of amino acid residues, potential binding sites, and structural motifs critical for protein function. Additionally, the predicted structure facilitated further computational analyses, such as protein-ligand interactions and virtual screening studies, aimed at identifying potential therapeutic compounds targeting IRS1.The 3D structure IRS1 was taken from PDB file under ID 1IRS [80].

The protein structures were ready in PyMOL by first water molecules were removed and any ligands present. Subsequently, to the structures missing polar hydrogens were added. The energy minimization process was then conducted to achieve stable conformations, thereby avoiding any overlaps within the structure. Finally, the updated structures were saved in PDB format. The refined structures are depicted in the image below, illustrating their optimized conformations after the preparation steps described above [82].

FIGURE 4.2: 3D Structure of IRS1

Above figure representing three-dimensional structure of Insulin Receptor Substrate 1 (IRS1) protien.

4.1.4 Identifying Functional Domains in Proteins

The database Interpro was utilized to determine the domains and functional locations of selected proteins. Interpro is a tool for functional study of protein sequences. Conserved domains play a role in sequencing, structure, and relationships.

Proteins can contain More than one functional domain performs various functions. Functional domains are active parts of proteins that involve in interaction of protien with other substances within the sequence range of 156 to 261 in IRS-1, there is primarily the Phosphotyrosine Binding (PTB) domain and Src homology 2 (SH2).

This domain is responsible for recognizing and binding to specific phosphotyrosine motifs on the activated insulin receptor [84].

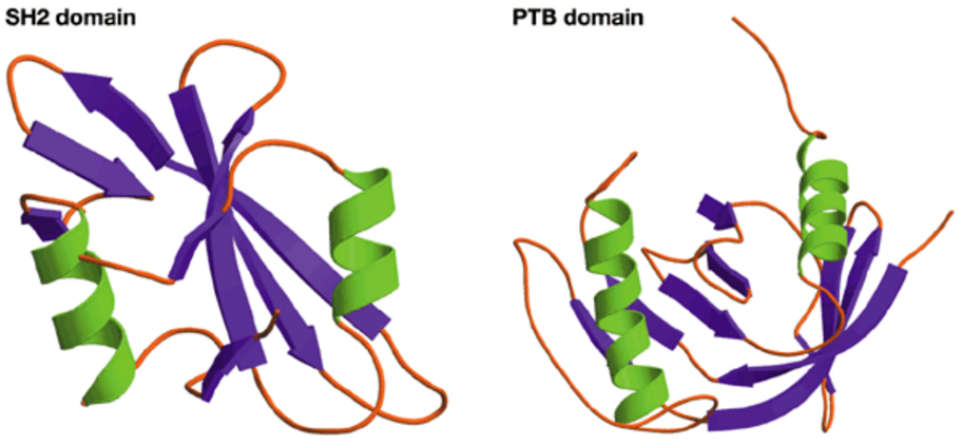


FIGURE 4.3: Figure showing functional domains

4.2 Ligand Selection

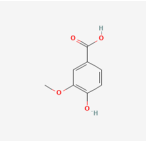
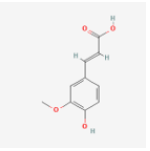
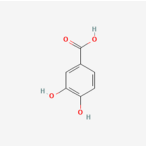
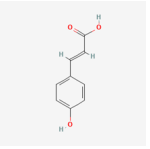
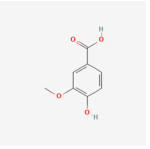
The Protein Data Bank (PDB) provides a massive collection of protein-ligand interactions that are critical for drug discovery and structural biology. The selection of ligands is based on high-resolution structures, relevant chemical classes, and strong binding affinities. Conformational selection, a crucial process in which a ligand binds and stabilizes a particular protein conformer, increases the population of that conformer, resulting in the development of stable complexes. Protein dynamics, ligand efficiency, structure-activity relationship (SAR) data, computational predictions, and functional assays are all used to optimize ligand selection, resulting in a complete strategy to identifying and refining effective protein-ligand interactions

Ligands were searched out from the chemical information database PubChem pubchem.ncbi.nlm.nih.gov [80]. The Protein Data Bank (PDB) provides global access to information on protein-ligand interactions, including 3D ligand structures available for download in SDF format from PubChem. Following the selection of ligands, Chem 3D software (version 12.0.2) is used to minimize energy. To prepare ligands for docking, follow the Lipinski rule of five: molecular weight $\leq$500, log P $\leq$5, hydrogen bond donors $\leq$5, and hydrogen bond acceptors $\leq$10. This step enhances the stability and reliability of docking data by avoiding unstable ligands

from producing unreliable Vina scores. Vanillic acid, Ferulic acid, Protocatechuic acid, P-coumaric acid, Ellagic acid were chosen as ligands in the current study all of which followed the Lipinski rule for orally active compounds [93].

These rules are to be followed by orally active compounds. The drug like is dependent on the mode of administration.

TABLE 4.2: The following table represents Structure of ligands

Ligand Name	**Molecular Formula**	**Molecular weight**	**Canonical Smiles**	**Structure**
Vanillic acid	$C_8H_8O_4$	168.15 g/-mol	COC1=C(C= CC(=C1)C(=O)O)O	
Ferulic acid	$C_{10}H_{10}O_4$	194.18 g/-mol	COC1=C(C= CC(=C1)C=CC(=O)O)O	
Protocatechuic acid	$C_7H_6O_4$	154.12 g/-mol	1=CC(=C(C= C1C(=O)O)O)O	
P-coumaric acid	$C_9H_8O_3$	164.16 g/-mol	C1=CC(=CC= C1C=CC(=O)O)O	
Ellagic acid	$C_{14}H_6O_8$	302.19 g/-mol	C1=C2C3=C(C(= C1O)O)OC(=O)C4=CC(= C(C(=C43)OC2=O)O)O	

4.3 Active Site Identification

CASTp program identifies active protein sites by predicting available pockets for binding and providing surface area and volume information. CASTp (Computed Atlas of Surface Topography of Proteins) is a computer method for identifying and characterizing the topography of protein structures. It contains extensive information about the pockets and cavities on the protein surface, which are necessary for understanding protein-ligand interactions, enzyme active sites, and other functional areas. CASTp examines protein structures to discover surface features and computes various geometric properties such as area, volume, and the number of accessible surface points [94].

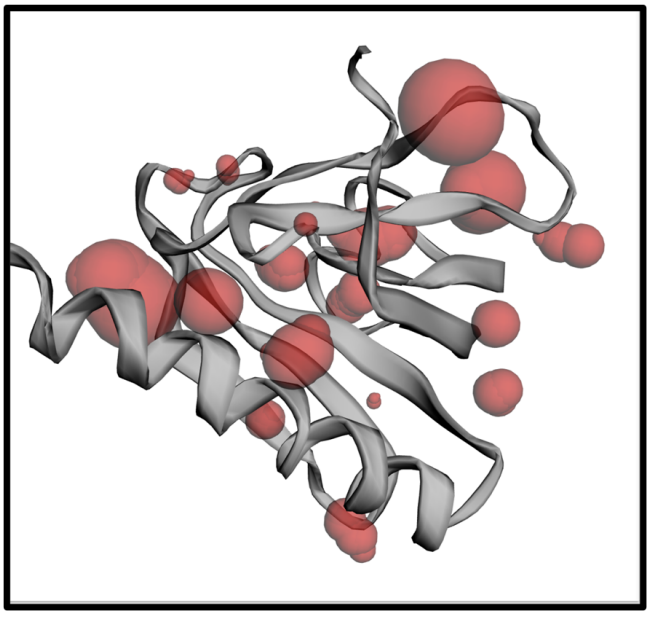


FIGURE 4.4: Structure of IRS1 protein showing available pockets for ligands.

The image above depicts the structure of the accessory gene regulator protein. The red color indicates the accessible binding pocket for protein. The binding pocket is an area where ligands can bind. The table above displays the number of pockets, their sizes, and volumes

TABLE 4.3: Area and volume of binding pockets of IRS

Pocket ID	**Area (SA)**	**Volume (SA)**
1	16.347	10.573

Table 4.3 continued from previous page

Pocket ID	Area (SA)	Volume (SA)
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	2.52	0.209
14	1.337	0.058
15	1.281	0.043
16	1.039	0.038
17	0.702	0.023
18	0.42	0.011
19	0.44	0.006
20	0.131	0.006
21	0.235	0.004
22	0.041	0.001
23	0.012	0

The table 4.3 above shows the binding pocket IDs, area, and volume of accessory gene regulator protein A. It indicates that there are twenty-three pockets available for protein IRS1. The largest binding pocket has a surface area of 27.133 and a volume of 5.969. The smallest pocket has a surface area of 0.012 and a volume of 0.000.

4.4 Ligand and Target Protein Interaction

Estimated the interaction between the ligand's active pockets and the protein to interpret docking results. Two types of interactions were investigated: hydrogen

bonding and hydrophobic bonding interactions. Ligplot Plus (version v.1.4.5) was used to study protein-ligand interactions [95]. Ligplot was used to identify the interaction between active confirmation ligands and target proteins. We evaluated the stored conformations of each molecule's ligand receptor complex in detail [95].

4.5 ADME/T Properties of Ligands

Lipinski's five-drug law is used to determine verbal bioavailability and artificial availability. In a second investigation, the ADME/T characteristics of ligands were calculated as a measure of pharmacokinetics using the online application pkCSM. Pharmacology includes two major terms: pharmacodynamics and pharmacokinetics.

In pharmacology, absorption is the process by which a medication moves from the bloodstream into tissues. The rate and extent of this absorption are influenced by the drug's chemical properties and its environment. For a drug to be absorbed, it must traverse cellular barriers, either epithelial or endothelial. Only a few drugs can actively cross these barriers, which requires energy to move against a concentration gradient [85].

Medication often moves from areas of high concentration to low concentration via passive diffusion through cell membranes. This process does not require energy but is influenced by the drug's size and solubility.

All ligands have low water solubility and poor skin absorption, but they typically exhibit permeability through CaCO2. Juglone, physcion, and coumarin absorb at rates above 90%, whereas emodin and resveratrol have average absorption rates, and the remaining ligands absorb very little. Skin permeability for all ligands is poor. Juglone has a negative value for P-glycoprotein (Pgp) substrate, while the other ligands show a positive value for one component. A positive Pgp substrate value means the chemical can be quickly pumped out of cells, reducing its absorption.

In pharmacology, distribution refers to the movement of drugs from one region of the body to another. One of the four ADME/T (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties is the volume of distribution in humans (VDss, measured in log L/kg). Other important factors include the human unbound fraction (Fu), blood-brain barrier permeability (log BB), and central nervous system permeability (log PS) [85].

4.5.1 Vanilic Acid

Vanillic acid's bioavailability is influenced by its solubility and the presence of other chemicals in the gastrointestinal tract. When taken orally, it is relatively well absorbed. It can pass through the intestinal epithelium and has moderate water solubility, facilitating absorption in the gastrointestinal tract. However, transport processes and interactions with intestinal enzymes may affect its absorption efficiency

4.5.1.1 Absorption Property

Table 4.4: Absorption properties of vanilic acid

Property	**Model Name**	**Predicted value**
Absorption	Water solubility	-1.838
	Caco2 permeability	0.33
	Intestinal absorption	78.152
	Skin permeability	-2.726
	P-glycoprotein subsrate	No
	P-glycoprotein I inhibitor	No
	P-glycoprotein II inhibitor	No

Protocatechuic acid has intermediate water dissolvability, with a log mol/L value of -1.838, indicating that it might disintegrate sufficiently in fluid conditions. It has modest permeability across intestinal epithelial cells, which is required for oral bioavailability, as evidenced by its Caco2 permeability of 0.33 log Papp in 10-6 cm. The high intestine absorption rate of 78.152% suggests that the gastrointestinal tract effectively transports the substance into the systemic

circulation. Furthermore, its skin permeability, as seen by a log Kp value of -2.726, shows poor dermal absorption, which, when given topically, can assist reduce systemic exposure.

4.5.1.2 Distribustion Property

Vanillic acid has a moderate distribution throughout the body's tissues and does not accumulate extensively in specific organs. It binds reasonably well to plasma proteins, which affects its free fixation and pharmacological accessibility. Its ability to traverse the blood-mind barrier is reduced, limiting its effects on the focused sensory system.

Table 4.5: Distribution properties of vanilic acid

Property	**Model Name**	**Predicted value**
Distribution	VDss (human)	-1.739
	Fraction unbound (human)	0.518
	BBB permeability	-0.38
	CNS permeability	-2.628

Protocatechuic corrosive has moderate dispersion throughout the body, as evidenced by a VDss of -1.739 (log L/kg). A substantial quantity remains in circulation in its active, unbound state, with a fraction unbound (Fu) of 0.518. It possesses moderate permeability to the central nervous system (log PS -2.628) and the ability to penetrate the blood-brain barrier (log BB -0.38). Understanding its systemic availability and its therapeutic applications is dependent on its distribution qualities.

4.5.1.3 Metabolism Property

Vanillic acid undergoes only a few phase I metabolic processes, mostly oxidation and reduction, which are catalyzed by enzymes such as cytochrome P450. Phase II conjugation processes, such as glucuronidation and sulfation, which make it more soluble and simpler to excrete, increase its metabolization potential. The principal

metabolites are vanillic acid glucuronide and vanillic acid sulfate, both of which are water-soluble and eliminated in urine.

TABLE 4.6: Metabolism properties of vanilic acid

Property	Model Name	Predicted value
Metabolism	CYP2D6 substrate	No
	CYP3A4 substrate	No
	CYP1A2 inhibitor	No
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	No
	CYP2C9 inhibitor	No
	CYP3A4 inhibitor	No

Protocatechuic acid is not expected to be a substrate for CYP2D6 or CYP3A4 compounds, nor will it inhibit CYP1A2, CYP2C19, CYP2C9, or CYP3A4 catalysts. Because of its excellent pharmacokinetic profile, these metabolic features indicate that protocatechuic acid is unlikely to severely impair the metabolism of other medicines processed by this enzyme.

4.5.1.4 Excretion Property

TABLE 4.7: Excretion properties of vanilic acid

Property	Model Name	Predicted value
Excretion	Total Clearance	No
	Renal OCT2 substrate	No

4.5.1.5 Toxicity Property

Vanillic acid has a low acute toxicity and is generally regarded safe, while large dosages may induce minor gastrointestinal discomfort. Although additional research is needed to properly understand its chronic toxicity profile, long-term use has not been associated with major side effects. There is no significant evidence that vanillic corrosive is genotoxic or carcinogenic, hence it is generally safe to use.

The security edge for vanillic corrosive is large, indicating that the beneficial portion is well beneath the poisonous portion, which improves its security for various applications.

TABLE 4.8: Toxicity properties of vanilic acid

Property	**Model Name**	**Predicted value**
Toxicity	AMES toxicity	No
	Max. tolerated dose (human)	0.719
	hERG I inhibitor	No
	hERG II inhibitor	No
	Oral Rat Acute Toxicity (LD50)	2.454
	Oral Rat Chronic Toxicity (LOAEL)	2.032
	Hepatotoxicity	No
	Skin Sensitation	No
	T.Pyriformis toxicity	0.265
	Minnow toxicity	1.926

Ellagic cacid exhibits potential security properties for anticipated restorative applications. It does not inhibit hERG I and II, indicating that it is not harmful to AMES or the heart. Furthermore, neither T. Pyriformis (0.265 log ug/L) nor minnows (1.926 log mM) are adversely affected by elagic acid, nor is their skin susceptible to it. These results suggest its potential as a safe option for future therapeutic research.

4.5.2 Ferulic Acid

Ferulic corrosive, a common phenolic chemical found in plants, exhibits a variety of ADMET (Retention, Conveyance, Digestion, Discharge, and Poisonousness) qualities that are important for determining its restorative ability. Ferulic acid's bioavailability is controlled by its solubility and the presence of other compounds in the gastrointestinal tract, both of which affect oral absorption. It is easier to absorb through the intestinal membrane since it is soluble in both water and fats. However, the effectiveness of its absorption varies according on intestinal permeability, interactions with dietary components, and gut microbes.

4.5.2.1 Absorption Property

TABLE 4.9: Absorption properties of ferulic acid

Property	Model Name	Predicted value
Absorption	Water solubility	-2.817
	Caco2 permeability	0.176
	Intestinal absorption	93.685
	Skin permeability	-2.72
	P-glycoprotein subsrate	No
	P-glycoprotein I inhibitor	NO
	P-glycoprotein II inhibitor	No

The compound's retention qualities reveal favorable attributes for a prospective medicine bid. With a log mol/L value of -2.817, it has a moderate water solubility and a significant intestinal absorption efficiency of 93.685. At 0.176 log Papp (10-6), Caco2 permeability is moderately favorable, indicating that it can pass through biological membranes. Importantly, it does not inhibit P-glycoproteins I or II and does not act as a substrate for P-glycoprotein, lowering the possibility of efflux transporter interference with absorption processes. In addition, the chemical has a log Kp of -2.72 and modest skin permeability. Together, these qualities imply that oral medication administration may be feasible.

4.5.2.2 Distribustion Property

Ferulic acid is dispersed throughout the body's tissues in a moderate volume following absorption, indicating that it does not accumulate extensively in any single organ. Ferulic acid binds reasonably to plasma proteins, influencing its free focus and accessibility for pharmacological effects. Its ability to permeate the blood-cerebrum barrier is limited, limiting its focal sensory system affects.

TABLE 4.10: Distribution properties of ferulic acid

Property	Model Name	Predicted value
Distribution	VDss (human)	-1.367
	Fraction unbound (human)	0.343

Table 4.10 continued from previous page

Property	Model Name	Predicted value
	BBB permeability	-0.239
	CNS permeability	-2.612

The compound's dispersion qualities imply potential medication application-friendly characteristics. It has a predicted VDss (human) value of -1.367 log L/kg, implying that it is dispersed modestly throughout the body. In human plasma, the fraction unbound (Fu) is 0.343, indicating that a significant portion of the chemical is still unbound and potentially active. The chemical has a BBB permeability of -0.239 log BB and a CNS permeability of -2.612 log PS, indicating considerable penetration into the central nervous system, which could be useful for neurodegenerative drugs. These circulatory parameters indicate that the drug may have reasonable pharmacokinetic qualities for restorative application.

4.5.2.3 Metabolism Property

Ferulic acid's extensive metabolism, mostly performed by phase II conjugation processes, produces the conjugated forms ferulic acid glucuronide and ferulic acid sulfate. These metabolites are more soluble in water, making them easier to defecate. Phase I metabolism is less essential, with fewer oxidation and reduction processes. Ferulic acid and its metabolites are predominantly eliminated by the kidneys, with conjugated metabolites excreting more efficiently due to their higher solubility. Ferulic acid has a short half-life, indicating that it is quickly eliminated from the body. As a result, it may need to be taken on a regular basis to maintain its therapeutic effects.

Table 4.11: Metabolism properties of ferulic acid

Property	Model Name	Predicted value
Metabolism	CYP2D6 substrate	No
	CYP3A4 substrate	No
	CYP1A2 inhibitor	No
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	No

Table 4.11 continued from previous page

Property	Model Name	Predicted value
	CYP2C9 inhibitor	No
	CYP3A4 inhibitor	No

According to the expected values, the chemical has no significant interactions with the major CYP enzymes. It is not a substrate for CYP2D6 or CYP3A4, and it does not inhibit CYP1A2, CYP2C19, CYP2C9, CYP2C2, or CYP3A4 proteins. These qualities indicate that these metabolic pathways have limited potential for drug-drug interactions, which could improve its safety profile and make it suitable for therapeutic usage while not significantly influencing the metabolism of co-administered medications.

4.5.2.4 Excretion Property

Ferulic acid and its metabolites are predominantly eliminated by the kidneys, with conjugated metabolites excreting more efficiently due to their higher solubility. Ferulic acid has a short half-life, indicating that it is quickly eliminated from the body. As a result, it may need to be taken on a regular basis to maintain its therapeutic effects.

Table 4.12: Excretion properties of ferulic acid

Property	Model Name	Predicted value
Excretion	Total Clearance	0.537
	Renal OCT2 substrate	No

The molecule has a modest elimination potential, with an estimated total clearance of 0.537 log ml/min/kg. Because it is not an OCT2 substrate, it appears to interact with the renal transport mechanism seldom. These discharge characteristics are beneficial for maintaining stable plasma fixations and lowering the risk of renal aggregation, potentially adding to its pharmacokinetic profile suitable for therapeutic uses.

4.5.2.5 Toxicity Property

Although high dosages of ferulic acid might produce minor gastrointestinaldiscomfort, it is typically considered safe with low acute toxicity. Despite the fact that more extensive research on chronic toxicity is needed to completely understand its safety profile, long-term use has not been associated to substantial adverse consequences. Ferulic acid is typically considered safe to take because there is no significant evidence that it is genotoxic or carcinogenic. Ferulic acid has a large margin of safety, which means that the therapeutic dose is significantly lower than the hazardous dose, making it suitable for a wide range of uses.

Table 4.13: Toxicity properties of ferulic acid

Property	**Model Name**	**Predicted value**
Toxicity	AMES toxicity	No
	Max. tolerated dose (human)	1.082
	hERG I inhibitor	No
	hERG II inhibitor	No
	Oral Rat Acute Toxicity (LD50)	2.282
	Oral Rat Chronic Toxicity (LOAEL)	2.065
	Hepatotoxicity	No
	Skin Sensitation	No
	T.Pyriformis toxicity	0.271
	Minnow toxicity	1.825

4.5.3 Protocatechuic Acid

Protocatechuic corrosive, a phenolic chemical found in several plants, exhibits a variety of ADMET (Retention, Dispersion, Digestion, Discharge, and Harmfulness) qualities that are critical for determining its therapeutic potential. When given orally, protocatechuic acid is usually well absorbed. Its solubility and the presence of other chemicals in the gastrointestinal tract affect its bioavailability. Its mild water dissolvability aids in its retention across the gastrointestinal wall, while its effectiveness may be influenced by interactions with digestive chemicals and transport devices.

4.5.3.1 Absorption Property

TABLE 4.14: Absorption properties of Protocatechuic acid

Property	Model Name	Predicted value
Absorption	Water solubility	-2.069
	Caco2 permeability	0.49
	Intestinal absorption	71.174
	Skin permeability	-2.727
	P-glycoprotein subsrate	No
	P-glycoprotein I inhibitor	No
	P-glycoprotein II inhibitor	No

With a predicted value of -2.069, the compound has moderate water solubility. With a log Papp value of 0.49 , it has good permeability across Caco-2 cells, indicating efficient absorption potential. It has a high absorption rate of 71.174% under simulated intestinal conditions. In addition, the compound has a log Kp value of -2.727, indicating that it is unable to penetrate the skin barrier. It's anything but a substrate for P-glycoprotein (P-gp), P-glycoprotein I, or P-glycoprotein II, demonstrating a low probability of connections with these vehicle proteins.

4.5.3.2 Distribution Property

Proteocatechuic acid is transported throughout the body's tissues after it is absorbed. Its modest dispersion means that it passes across tissues without collecting in any particular organ. It binds well to plasma proteins, influencing its free fixation and pharmacological accessibility. It has minimal effects on the central nervous system since it does not penetrate the blood-brain barrier.

TABLE 4.15: Distribution properties of protocatechuic acid

Property	Model Name	Predicted value
Distribution	VDss (human)	-1.298
	Fraction unbound (human)	0.648
	BBB permeability	-0.683
	CNS permeability	-3.305

The majority of protocatechuic acid metabolism in the body occurs through phase II conjugation processes. It is broken down into a range of conjugated molecules,

including protocatechuic acid glucuronide and sulfate, which are more soluble in water and aid in excretion. Procatechuic acid's oxidation and reduction processes in phase I metabolism are less relevant.

4.5.3.3 Metabolic Properties

TABLE 4.16: Metabolism properties of protocatechuic acid

Property	**Model Name**	**Predicted value**
Metabolism	CYP2D6 substrate	No
	CYP3A4 substrate	No
	CYP1A2 inhibitor	No
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	No
	CYP2C9 inhibitor	No
	CYP3A4 inhibitor	No

The substance is not expected to be a substrate for CYP2D6 or CYP3A4 compounds, and it does not inhibit CYP1A2, CYP2C19, CYP2C9, or CYP3A4 catalysts. These findings suggest potential metabolic stability and a lower risk of drug-drug interactions mediated by cytochrome P450 enzymes. These data also indicate that there is a limited possibility of metabolic interactions between these enzymes.

4.5.3.4 Excretion Properties

The kidneys are the principal organs for eliminating protocatechuic acid and its metabolites. Conjugated metabolites are more efficiently excreted due to their enhanced solubility. Proteocatechuic acid has a short half-life, indicating that it is quickly eliminated from the body. To sustain therapeutic results, it may need to be administered on a regular basis.

TABLE 4.17: Excretion properties of protocatechuic acid

Property	**Model Name**	**Predicted value**
Excretion	Total Clearance	0.551
	Renal OCT2 substrate	No

The compound's anticipated total clearance of 0.551 indicates that it will be rapidly removed from the body. It is not identified as a substrate for renal OCT2

carriers, implying a low connection potential in renal discharge routes. These findings support its favorable pharmacokinetic profile, which stresses its rapid clearance from the body and minimal interaction with the kidney, both of which are desirable characteristics for drug development.

4.5.3.5 Toxicity Properties

The acute toxicity of protocatechuic acid is minimal. It is usually considered safe, despite the likelihood of moderate gastrointestinal discomfort at higher doses. Although there have been few extensive studies on chronic toxicity, and more study is needed to properly understand its safety profile, long-term use has not been associated with major side effects. There is no significant evidence that protocatechuic corrosive is genotoxic or causes cancer, hence it is generally safe to use. Proteocatechuic acid has a wide margin of safety, which means that the therapeutic dose is significantly lower than the dangerous dose, improving its application safety.

Table 4.18: Toxicity properties of protocatechuic acid

Property	**Model Name**	**Predicted value**
Toxicity	AMES toxicity	No
	Max. tolerated dose (human)	0.814
	hERG I inhibitor	No
	hERG II inhibitor	No
	Oral Rat Acute Toxicity (LD50)	2.423
	Oral Rat Chronic Toxicity (LOAEL)	2.021
	Hepatotoxicity	No
	Skin Sensitation	No
	T.Pyriformis toxicity	0.273
	Minnow toxicity	2.451

The AMES test confirms that the chemical has no mutagenic potential and shows promising safety features. The maximum tolerated dose in humans is anticipated to be 0.814 indicating a broad therapeutic window. It does not block hERG I or II channels, which are essential for heart protection. It has an acute LD50 of

2.423 mol/kg and a chronic LOAEL of 2.021 log mg/kg_bw/day in oral rat tests, indicating moderate toxicity. In addition, there is no evidence of hepatotoxicity, cutaneous sensitization, or aquatic toxicity. These findings indicate that it may be a safe option for future research.

4.5.4 P-coumaric Acid

4.5.4.1 Absorption Properties

TABLE 4.19: Absorption properties of P-coumaric acid

Property	**Model Name**	**Predicted value**
Absorption	Water solubility	-2.378
	Caco2 permeability	1.21
	Intestinal absorption	93.494
	Skin permeability	-2.715
	P-glycoprotein subsrate	No
	P-glycoprotein I inhibitor	No
	P-glycoprotein II inhibitor	No

P-coumaric acid has several key absorption qualities that must be considered in order to fully understand its therapeutic potential. It has a value of -2.378, indicating weak aqueous solubility. However, the projected Caco2 permeability of 1.21 (log Papp in 10-6 cm/s) indicates that it can successfully permeate the intestinal epithelial barrier. Furthermore, P-coumaric acid has a high likelihood of absorption by the intestinal tract (93.494%), showing that it is well-absorbed by the human digestive system. With a log Kp value of -2.715, the compound's skin permeability is also low, indicating minimal absorption through the skin. Importantly, P-coumaric acid does not act as a P-glycoprotein substrate or inhibitor. This indicates that P-glycoprotein transporters are unlikely to actively efflux it, potentially affecting its bioavailability and distribution throughout the body.

4.5.4.2 Distribition Properties

TABLE 4.20: Distribition properties of P-coumaric acid

Property	Model Name	Predicted value
Distribution	VDss (human)	-1.151
	Fraction unbound (human)	0.428
	BBB permeability	-0.225
	CNS permeability	-2.418

P-coumaric acid exhibits excellent dispersion qualities, which are critical for determining its pharmacokinetics throughout the human body. The volume of dispersion (VDss) in humans is expected to be -1.151 , indicating a moderately low conveyance in tissues. The fraction unbound in human plasma (Fu) is 0.428, indicating that over 50% of the chemical remains unbound and can exert its pharmacological effects. Its penetrability across the blood-brain barrier (BBB) is expected to be - 0.225 log BB, indicating a limited ability to enter the focused sensory system (CNS). Furthermore, its CNS porousness is measured at -2.418 log PS, indicating a negligible admission into the mind. These dispersion features include P-coumaric corrosive's limited ability to penetrate into the CNS, which may impair its useful applications.

4.5.4.3 Metabolism Properties

TABLE 4.21: Metabolism properties of protocatechuic acid

Property	Model Name	Predicted value
Metabolism	CYP2D6 substrate	No
	CYP3A4 substrate	No
	CYP1A2 inhibitor	No
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	No
	CYP2C9 inhibitor	No
	CYP3A4 inhibitor	No

The digesting features of P-coumaric disclose its interactions with several cytochrome P450 (CYP) molecules that are essential for medication absorption.

P-coumaric corrosive is unlikely to be used by CYP2D6 or CYP3A4, as it is not a substrate for either enzyme. Additionally, it does not inhibit CYP3A4, CYP2C19, or CYP1A2. P-coumaric acid may have a decreased risk of medication interactions because it is not associated with these major CYP molecules, which is beneficial to its health profile and therapeutic application. These metabolic features are critical for understanding how the body processes P-coumaric acid and predicting how it will interact with other drugs.

4.5.5 Excretion Properties

Table 4.22: Excretion properties of P-coumaric acid

Property	**Model Name**	**Predicted value**
Excretion	Total Clearance	0.662
	Renal OCT2 substrate	No

The excretion qualities of P-coumaric acid show how the chemical is removed from the body. The anticipated total clearance rate of 0.662 log ml/min/kg indicates how quickly P-coumaric acid leaves the bloodstream. It is also improbable that the renal organic cation transporter 2 (OCT2) will actively transport it because it is not a substrate. Because of these features, P-coumaric acid has a modest clearance rate and relies less on renal OCT2-mediated processes for elimination, both of which might affect the drug's overall pharmacokinetics and duration of action in the body.

4.5.5.1 Toxicity Properties

Table 4.23: Toxicity properties of P-coumaric acid

Property	**Model Name**	**Predicted value**
Toxicity	AMES toxicity	No
	Max. tolerated dose (human)	1.111
	hERG I inhibitor	No
	hERG II inhibitor	No
	Oral Rat Acute Toxicity (LD50)	2.155

Table 4.23 continued from previous page

Property	Model Name	Predicted value
	Oral Rat Chronic Toxicity (LOAEL)	2.534
	Hepatotoxicity	No
	Skin Sensitation	No
	T.Pyriformis toxicity	0.319
	Minnow toxicity	1.607

4.5.6 Ellagic Acid

Ellagic acid is a naturally occurring polyphenolic chemical present in a wide variety of fruits and vegetables. It possesses a number of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) qualities that are critical for determining how it might be employed in medicine. Ellagic corrosive has poor oral absorption due to its poor water solubility and limited gastrointestinal porousness. Its bioavailability is entirely influenced by interactions with food components and stomach microbes, which can convert it into more absorbable compounds such as urolithins.

4.5.6.1 Absorption Properties

Table 4.24: Absorption properties of Ellagic acid

Property	Model Name	Predicted value
Absorption	Water solubility	-3.181
	Caco2 permeability	0.335
	Intestinal absorption	86.684
	Skin permeability	-2.735
	P-glycoprotein subsrate	Yes
	P-glycoprotein I inhibitor	No
	P-glycoprotein II inhibitor	No

Ellagic acid exhibits unique ingesting qualities that are important for its bioavailability and therapeutic efficacy. The projected water solubility of -3.181 log mol/L

suggests that it is poorly soluble in water, which may impede digestion and absorption.

The Caco2 permeability score of 0.335 log Papp indicates moderate permeability through intestinal epithelial cells. It is expected to have an intestinal absorption rate of 86.684 percent, which indicates a rather high absorption rate in the human gut. The skin permeability is expected to be -2.735, indicating minimal absorption via the skin. Because ellagic acid is a P-glycoprotein substrate, it can be actively transported out of cells, potentially reducing its concentration within them.

4.5.6.2 Distribustion Properties

Table 4.25: Distribition properties of Ellagic acid

Property	**Model Name**	**Predicted value**
Distribution	VDss (human)	0.375
	Fraction unbound (human)	0.083
	BBB permeability	-1.272
	CNS permeability	-3.533

Ellagic acid's unique distribution characteristics have an impact on pharmacokinetics and medicinal efficacy. The volume of circulation (VDss) in people is assessed to be 0.375 log L/kg, showing impressive appropriation across the body's tissues. A small amount of ellagic acid remains unbound to plasma proteins, affecting its bioavailability and ability to reach target locations, as evidenced by the 0.083 percent unbound percentage in human plasma (Fu).

The blood-cerebrum boundary (BBB) penetrability is assessed to be - 1.272 , showing negligible entrance into the mind and in this manner restricted impacts on the focal sensory system (CNS). Additionally, its estimated CNS permeability of -3.533 indicates a limited ability to enter the CNS. These dissemination highlights are pivotal to understanding the pharmacological idea of ellagic.

4.5.6.3 Metabolism Properties

TABLE 4.26: Metabolism properties of Ellagic acid

Property	Model Name	Predicted value
Metabolism	CYP2D6 substrate	No
	CYP3A4 substrate	No
	CYP1A2 inhibitor	Yes
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	No
	CYP2C9 inhibitor	No
	CYP3A4 inhibitor	No

The metabolism qualities highlight its interaction with a variety of CYP enzymes, which are required for drug metabolism. Ellagic acid is not metabolized by CYP2D6 or CYP3A4, as these enzymes do not recognize it as a substrate. However, because it inhibits CYP1A2, it may influence how other medicines produced by this enzyme are metabolized. Because it does not affect CYP2C19, CYP2C9, or CYP3A4, it is unlikely to interact with medications processed by these enzymes. Understanding these metabolic features is crucial for predicting potential drug-drug interactions and optimizing ellagic acid's medicinal usage.

4.5.6.4 Excretion Properties

TABLE 4.27: Excretion properties of Ellagic acid

Property	Model Name	Predicted value
Excretion	Total Clearance	0.537
	Renal OCT2 substrate	No

The excretion qualities show how the chemical is removed from the body. The total clearance rate, 0.537, represents how efficiently ellagic acid is removed from the system. This proposes a moderate rate of liberation, implying that the construct remains in the body for a set period before being discharged. Furthermore, the renal OCT2 transporter does not have ellagic acid as a substrate, hence it is doubtful that this pathway actively transports it in the kidneys. Understanding

the excretion qualities of ellagic acid makes it easier to predict the duration and degree of its therapeutic effects, as well as potential side effects.

4.5.6.5 Toxicity Properties

Table 4.28: Toxicity properties of Ellagic acid

Property	**Model Name**	**Predicted value**
Toxicity	AMES toxicity	No
	Max. tolerated dose (human)	0.476
	hERG I inhibitor	No
	hERG II inhibitor	No
	Oral Rat Acute Toxicity (LD50)	2.399
	Oral Rat Chronic Toxicity (LOAEL)	2.698
	Hepatotoxicity	No
	Skin Sensitation	No
	T.Pyriformis toxicity	0295
	Minnow toxicity	2.11

A negative AMES toxicity result suggests that ellagic acid's toxicity profile is modest in terms of inducing genetic abnormalities. For humans, the maximum tolerated dose is 0.476 log mg/kg/day, indicating a moderate dosage threshold above which deleterious effects may occur. It does not inhibit the hERG I or hERG II channels, indicating that it is safe for producing heart arrhythmias. The oral rat acute toxicity (LD50) value of 2.399 mol/kg represents the fatal dose for half of the tested rat population. Persistent poisonousness in rodents has a LOAEL of 2.698 log mg/kg_bw/day, indicating the smallest part at which adverse effects are observed. Ellagic acid is not harmful to the liver and does not increase skin sensitivity.

4.6 Lipinski's Rule of Five

Lipinski's Rule of Five outlines the following criteria for "drug-like" molecules:

1. The logP value should be less than or equal to 5.

2. The molecular weight should be under 500 daltons.

3. The maximum number of hydrogen bond acceptors should be 10.

4. Maximum number of H-bond donor should be 5

As a result, our compound is run according to the guidelines, allowing for the study of various binders of *Momordica charantia* checked and results are shown in table 4.29.

TABLE 4.29: Study of various binders of *Momordica charantia*

Ligands	**logP Value**	**Molecular Weight**	**H-bond Acceptor**	**H-bond Donor**
Vanillic acid	1.099	168.148	3	2
Ferulic acid	1.4986	194.186	3	2
Protocatechuic acid	0.796	154.121	3	3
P-coumaric acid	1.49	164.16	2	2
Ellagic acid	1.3128	302.194	8	4

These properties provide insight into the chemical characteristics of each ligand, including their lipophilicity (logP), molecular weight, and their ability to participate in hydrogen bonding interactions (H-bond acceptors and donors). These factors are crucial in understanding their potential biological activities and pharmacological properties.

The table shows the hydrogen bond acceptor and donor, as well as the molecular weight and logP values. Values of Momordica charantia ligands. These requirements will be followed by orally active compounds. The drug-like impact is dependent on how it is consumed. A substance is deemed a medicine if it follows three or more rules; if it violates two or more requirements, it is thought to be poorly known. Aside from hyperoside and coumarin, almost every ligand followed the Lipinski standard of five.

4.7 Molecular Docking

Molecular docking is a computer technique for predicting the optimal orientation and binding affinity of a small molecule (ligand) when it interacts with a bigger biomolecule, most commonly a protein. This strategy is commonly used in drug discovery and development to identify new drug candidates and better understand their interactions with target proteins. The procedure begins with the creation of 3D structures of the target protein and ligand, which are often retrieved from databases such as the Protein Data Bank (PDB) for proteins and PubChem for ligands. Energy minimization and optimization techniques are used to guarantee that the structures are in the proper conformation for docking. Using known active sites, allosteric sites, or predictions using various computational tools.

During the docking simulation, the ligand is methodically placed in the protein's binding site in different orientations and conformations. The docking algorithm evaluates each pose using a scoring formula that estimates the ligand-protein complex's binding affinity. Poses are evaluated and ranked based on projected binding affinity, taking into account hydrogen bonding, hydrophobic interactions, electrostatic interactions, and steric complementarity. The top-scoring poses are then examined to better understand the binding interactions, identify critical residues involved in binding, and assess the stability and specificity of the ligand-protein complex.

Molecular docking provides insights into the molecular basis of ligand binding and aids in the rational design of new compounds with improved efficacy and selectivity. It is a crucial step in the early stages of drug discovery, allowing researchers to screen large libraries of compounds and identify promising candidates for further experimental validation. This method ensures that only stable and reliable ligands are used for docking studies to predict their binding affinities and interactions with target proteins, thereby facilitating the identification and optimization of effective drug candidates.

CB dock uses a novel rotation cavity detection approach to forecast and estimate a protein's binding site, as well as compute its center and size. Docking performed

using IRS1 protien and vanillic acid, ferulic acid, protocatechuic acid, P-coumaric acid, ellagic acid as ligand . Ligands with best binding score values with target proteins were represented in table. CB Dock, a user-friendly blind docking web server, was used to forecast binding modes without specific binding site information. CB dock gives fifive best interacting confifirmations for each ligand molecule.

In molecular docking, the conformations of the ligand-protein complexes are arranged based on their binding affinities. The optimal conformation is chosen based on the highest affinity score, which indicates the strength of the protein-ligand interaction. After the docking process, the resulting docked structures are selected for further analysis. The best-docked structure is identified by evaluating docking scores, cavity sizes, grid maps, and binding energies. These criteria help in determining the most promising ligand-protein interaction for subsequent studies.

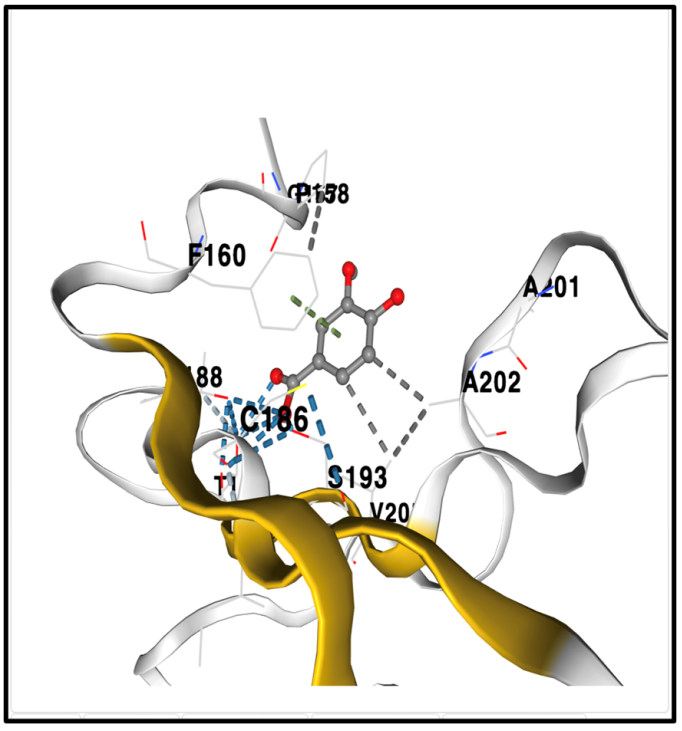


FIGURE 4.5: Dock complex of vanilic acid and IRS1

Both the standard and the lead compound were docked against the target proteins and the docking result gives us the best binding score

These conformations were ranked according to their binding affinities, and the best conformation was selected based on the highest affinity score of the protein-ligand interaction. After the docking process, the docked structures were chosen for further analysis. The best-docked structure was identified by evaluating the docking score, cavity size, grid map, and binding energy.

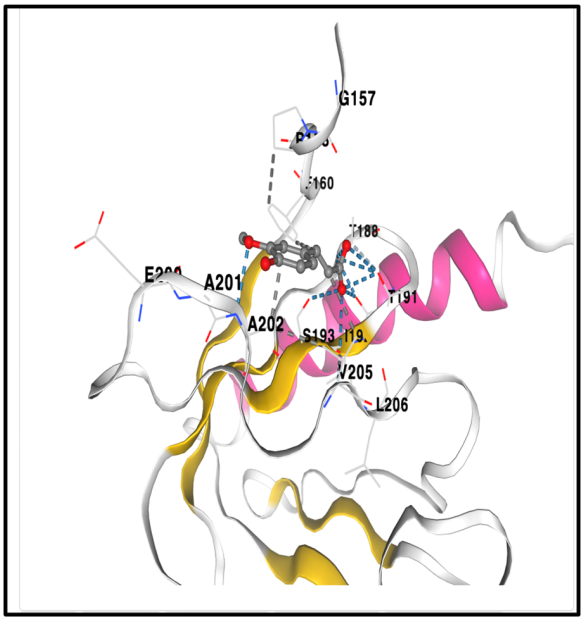


FIGURE 4.6: Dock complex of ferulic acid and IRS1

Both the standard and the lead compound were docked against the target proteins and the docking result gives us the best binding score

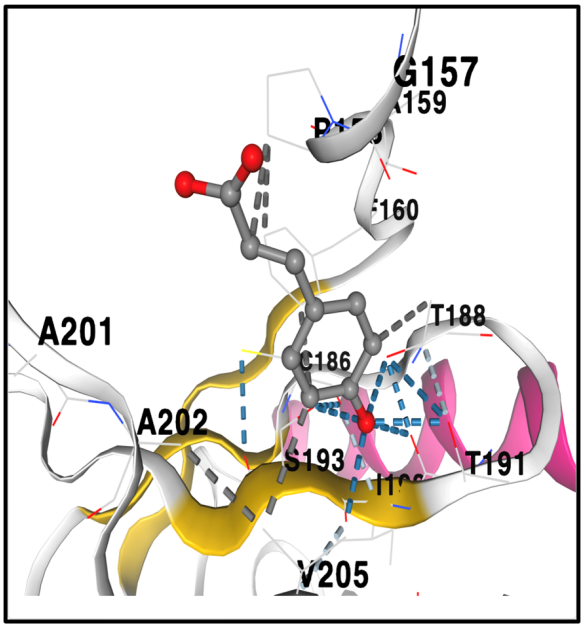


FIGURE 4.7: Dock complex of P-coumeric acid and IRS1

The best binding score was obtained when the standard and lead compounds were docked with the target proteins.

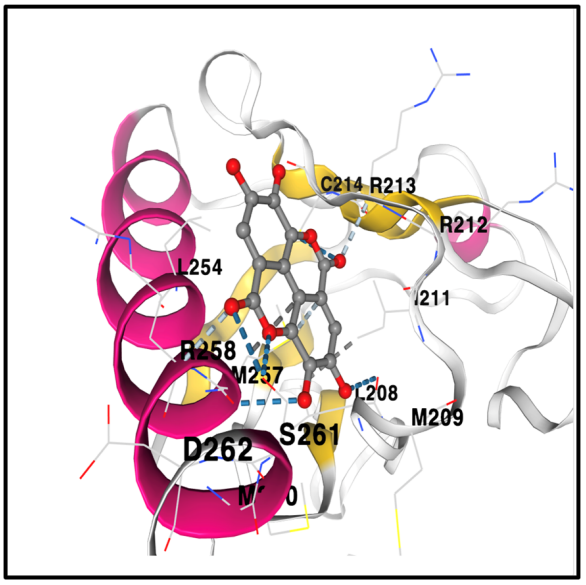


FIGURE 4.8: Dock complex of ellagic acid and IRS1

Docking the standard and lead compounds with the target proteins resulted in the highest binding score.

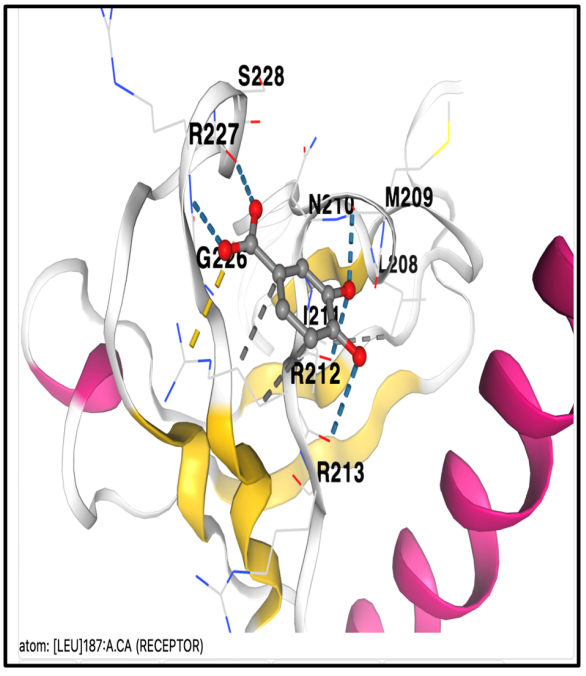


FIGURE 4.9: Dock complex of Protocatechuic acid and IRS1

Docking the standard and lead compounds with the target proteins resulted in the highest binding score.

TABLE 4.30: Results of CB dock with ligands name, vina score, centre volume, center, docking

Protien	**Ligand**	**Vina Score**	**Cavity Volume**	**Centre (x, y, z)**	**Docking size (x, y, z)**
IRS1	Protocatechuic acid	-4.7	92	-8, -1, -6	17, 17, 17
	P-coumaric acid	-4.8	67	2, 10, 8	19, 19, 19
	Ellagic acid	-6.2	92	-8, -1, -6	19, 19, 19
	Ferulic acid	-5.2	67	2, 10, 8	19, 19, 19
	Vanillic acid	-4.6	67	2, 10, 8	17, 17, 17

4.8 Ligplot

Ligplot is a popular computational tool for creating schematic diagrams of protein-ligand interactions. It gives a clear and precise picture of the interactions between a protein and a ligand, making it easier to analyse and comprehend the binding mechanisms.

LIGPLOT depicts hydrogen bonding and hydrophobic interactions, which are critical to the stability and selectivity of the protein-ligand complex. This software generates schematic representations of protein-ligand interactions from specified ligands in PDB files.

The docked files were uploaded in PDB format to analyze hydrogen and hydrophobic bonds. A large number of hydrophobic and hydrogen bond interactions were found between the 10 ligands and the four target proteins.

Ligand-receptor complexes have significant hydrogen bonding and hydrophobic interactions. The following diagrams depict the ligand-receptor interactions.

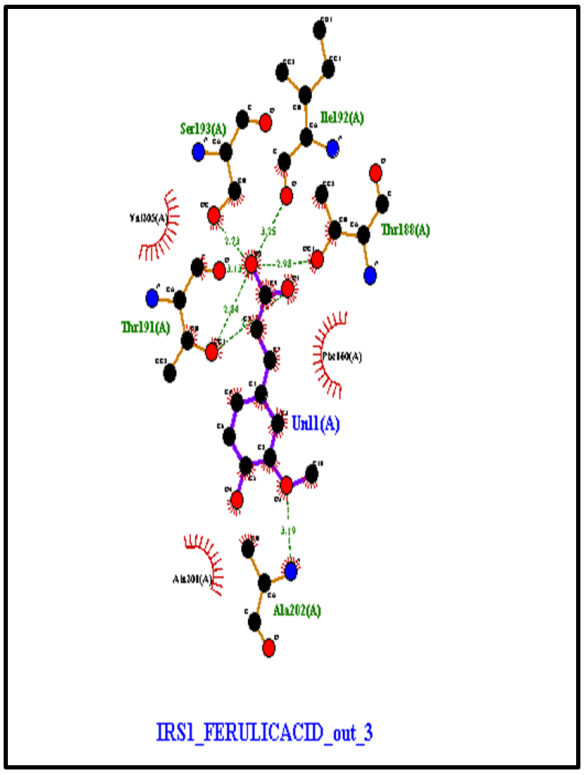


FIGURE 4.10: Interactions of ferulic acid by ligplot

Above figure 4.10 shows the interaction of ferulic acid with protien. it contain five amino acids and three hydrophobic interactions with seven hydrogen bonds.

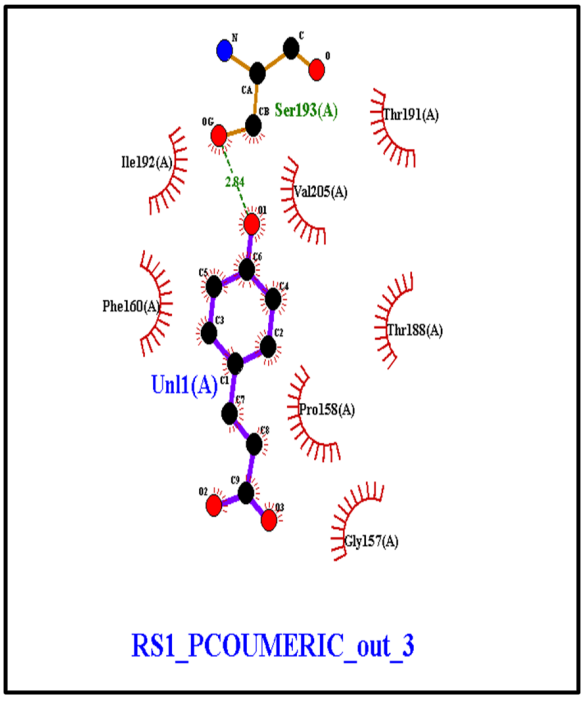


FIGURE 4.11: Interactions of P coumeric by ligplot

Figure 4.11 shows the interaction of P-coumeric with protien. It contain one amino acid and seven hydrophobic interactions and one hydrogen bond.

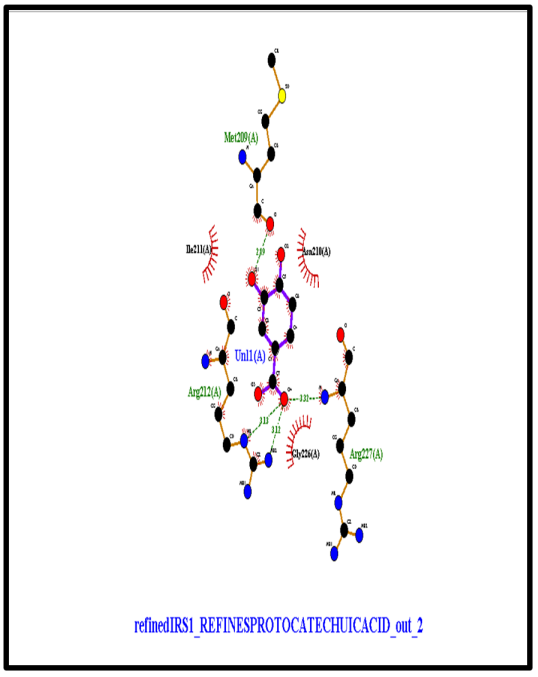


FIGURE 4.12: Interactions of Protocatechuic acid by ligplot

Figure 4.12 shows the interaction of Protocatechuic acid with protien. It contain three amino acid and three hydrophobic interactions and four hydrogen bond.

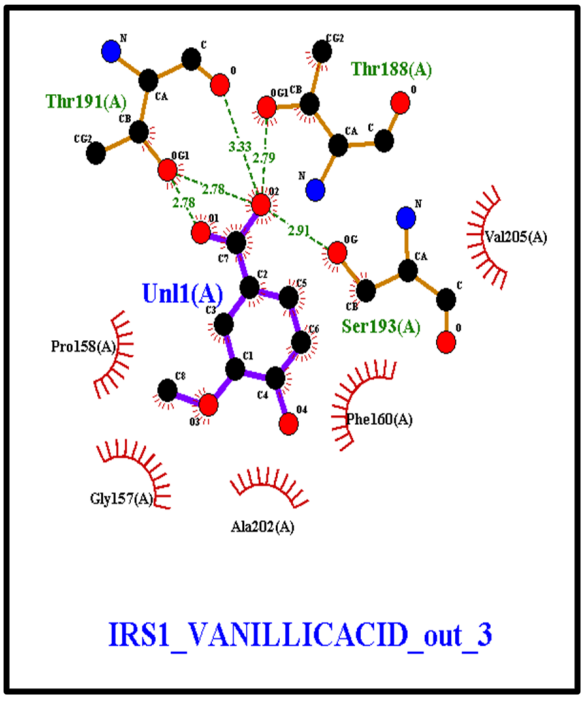


FIGURE 4.13: Interactions of vanillic acid by ligplot

Figure 4.13 shows the interaction of vanillic acid with protien. It contain three amino acid and five hydrophobic interactions and five hydrogen bond.

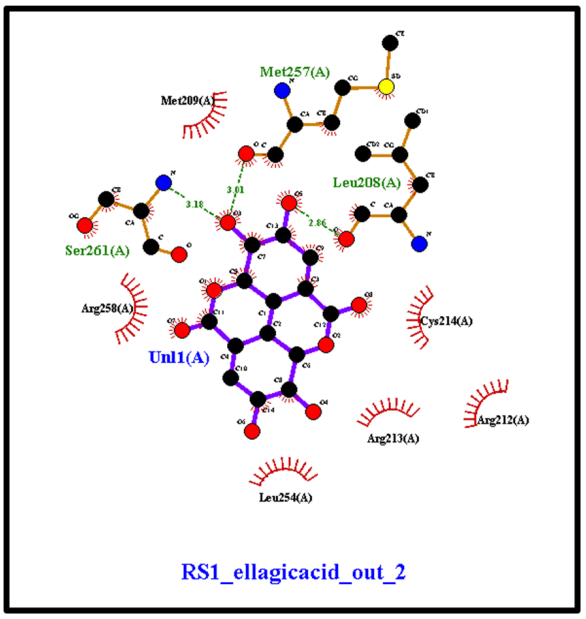


FIGURE 4.14: Interactions of ellagic acid by ligplot.

Figure 4.14 shows the interaction of ellagic acid with protien. It contain three amino acid and six hydrophobic interactions and three hydrogen bond.

4.9 Lead Compound Identification

Considering aspects such as absorption, distribution, metabolism, excretion, and toxicity in addition to the specified ADMET properties:

Protocatechuic acid and P-Coumaric have extremely similar ADMET profiles to Vanillic and Ferulic acids. These characteristics make them possible lead compounds, especially given their high solubility, retention, and dispersion properties.

Ellagic Acid has unique features, including the capacity to inhibit CYP1A2 and act as a P-glycoprotein substrate, both of which may be beneficial or detrimental depending on the therapeutic goals at hand. Its modest overall profile implies that it could be a lead drug, especially if P-glycoprotein substrate activity is considered beneficial.

Protocatechuic Acid appears as a promising lead chemical based on its ADMET properties,Retention, It has excellent water dissolvability, moderate Caco2 penetration, and high digestive absorption, indicating efficient uptake from the GI tract. Protocatechuic Acid has a moderate VDss (volume of distribution in humans) and a moderate unbound percentage, indicating that it is well distributed in biological tissues. Metabolism: Because it is not a substrate or inhibitor of key CYP enzymes, it is unlikely to interact with other metabolic enzymes. Excretion: Although no particular clearance statistics are available, its overall profile indicates a moderate clearance, which is generally advantageous for pharmacokinetics.Toxicity: The existing data show no substantial toxicity issues, giving it a safer candidate for therapeutic development. P-glycoprotein Substrate: Unlike the other chemicals studied, it is not a P-glycoprotein substrate, which may benefit cellular transport and bioavailability. Protocatechuic Acid has a balanced ADMET profile in light of these factors, making it a good candidate for further research and development as a lead molecule in pharmacological or therapeutic applications.

4.9.1 Reference Drug Identification

The reference drug selected on basis of physiochemical, ADMET properties, mode of action, and side effects. PubChem database used for physiochemical properties and pkCSM program used for ADMET properties of medicines. The Drug Bank and KEGG databases were used to determine the mechanism of action.

4.9.2 Metformin

Metformin is considered a cornerstone in diabetes research and treatment due to its dual effectiveness in lowering blood glucose levels and enhancing insulin sensitivity, making it indispensable for managing type 2 *diabetes mellitus* (T2DM). Its mechanism of action involves activating AMP-activated protein kinase (AMPK), which reduces hepatic glucose production and increases glucose uptake in peripheral tissues. This approach not only addresses hyperglycemia but also supports

modest weight loss and cardiovascular health. With a well-established safety profile and minimal risk of hypoglycemia, Metformin is widely prescribed for long-term diabetes management, underscoring its role as a standard against which new antidiabetic therapies and natural compounds are frequently evaluated.

4.9.3 Metformin Mechanism of Action

Metformin, a popular medication for type 2 diabetic mellitus (T2DM), acts by decreasing hepatic glucose production and boosting insulin sensitivity, but it does more. The principal action of this enzyme is to activate AMP-activated protein kinase (AMPK), which is necessary for cellular energy regulation. Metformin suppresses gluconeogenesis in the liver, lowering glucose production, by activating AMPK. Furthermore, Metformin increases glucose absorption in peripheral tissues, particularly muscles, and improves insulin sensitivity. Glycemic control improves, and blood glucose levels fall as a result of this dual impact. In addition to its cardiovascular benefits, Metformin induces mild weight loss and improves lipid metabolism. With a well-established safety profile and a low risk of causing hypoglycemia, Metformin is frequently considered the first-line treatment for T2DM and serves as a standard for evaluating new antidiabetic drugs.

4.9.4 Drug ADMET Properties

4.9.4.1 Absorption Properties Comparison

Table 4.31: Absorption properties

Property	**Model Name**	**Predicted value of Metformin**	**Predicted value of Protocatechuic acid**
Absorption	water solubility		-2.069
	Caco2 permeability	-5.24	0.49
	Intestinal absorption	Absorbed (high confidence)	71.174
	Skin permeability	2.96	-2.727

Table 4.31 continued from previous page

Property	Model Name	Predicted value of Metformin	Predicted value of Protocatechuic acid
	P-glycoprotein subsrate	substrate (high confidence)	No
	P-glycoprotein I inhibitor	Non inhibitor	No
	P-glycoprotein II inhibitor	Non inhibitor	No

The chemical possesses moderate water solubility and high permeability through the Caco-2 cell line, as evidenced by its absorption characteristics. The compound's intestinal absorption and skin permeability have been confirmed to be high. It is also known as a P-glycoprotein substrate, but it does not inhibit P-glycoprotein I or II.

4.9.4.2 Distribution Properties Comparison

TABLE 4.32: Distribution properties

Property	Model Name	Predicted value of Metformin	Predicted value Protocatechuic acid
Distribution	VDss (human)	1.22	-1.298
	Fraction unbound (human)	0.78	0.648
	BBB permeability	Penetrable	-0.683
	CNS permeability	-3.16	-3.305

The compound's dispersion parameters indicate a volume of dissemination (VDss) of 1.22 log L/kg and a fraction unbound in humans of 0.78. The chemical has a CNS permeability log PS value of -3.16 and can pass through the blood-brain barrier (BBB).

4.9.4.3 Metabolism Properties Comparison

Table 4.33: Metabolism properties

Property	**Model Name**	**Predicted value of Metformin**	**Predicted value Protocatechuic acid**
Metabolism	CYP2D6 substrate	No	No
	CYP3A4 substrate	No	No
	CYP1A2 inhibitor	No	No
	CYP2C19 inhibitor	No	No
	CYP2C9 inhibitor	No	No
	CYP2C9 inhibitor	No	No
	CYP3A4 inhibitor	No	No

The compound's metabolic characteristics indicate that neither the CYP2D6 nor the CYP3A4 enzymes use it as a substrate. Furthermore, it does not affect the CYP3A4 or CYP1A2 enzymes. This suggests that the molecule may have a limited potential for metabolic interactions with these substances.

4.9.4.4 Excretion Properties Comparison

Table 4.34: Excretion properties

Property	**Model Name**	**Predicted value of Metformin**	**Predicted value Protocatechuic acid**
Excretion	Total Clearance	5	0.551
	Renal OCT2 substrate	No	No

The total clearance is expected to be 5.00 log ml/min/kg based on estimated excretion parameters. This shows how well the chemical is removed from the body. Because the molecule is not a substrate for the kidney's OCT2 transporter, it is unlikely that it is removed by this pathway. This information is required to understand the compound's excretion profile and potential accumulation inside the body, both of which affect its overall pharmacokinetics and safety.

4.9.4.5 Toxicity Properties Comparison

TABLE 4.35: Toxicity properties

Property	**Model Name**	**Predicted value of Metformin**	**Predicted value Protocatechuic acid**
Toxicity	AMES toxicity	yes	No
	Max. tolerated dose (human)	1.7	0.814
	hERG I inhibitor	No	No
	hERG II inhibitor	No	No
	Oral Rat Acute Toxicity (LD50)	2.12	2.423
	Oral Rat Chronic Toxicity (LOAEL)	2.09	2.021
	Hepatotoxicity	No	No
	Skin Sensitation	No	No
	T.Pyriformis toxicity	3.05	0.273
	Minnow toxicity	5.08	2.451

It suggests possible mutagenesis consequences and is positive for AMES toxicity. The maximum tolerated portion in humans is expected to be 1.70 log mg/kg/day.

There is minimal risk of cardiac damage because the chemical does not block hERG I or hERG II. The acute toxicity (LD50) and chronic toxicity (LOAEL) for the oral rat are 2.09 log mg/kg_bw/day and 2.12 mol/kg, respectively. The chemical is safe for the liver and does not cause skin irritation.

Finally, the T. The Pyriformis toxicity of 3.05 log ug/L and the Minnow toxicity of 5.08 log mM provide a thorough evaluation of its possible toxicological profile.

4.9.5 Docking Score Comparison

The target proteins were docked against both the standard and lead compounds. The docking result provides us with the optimal binding score.

Table 4.36 demonstrates that Protocatechuic acid, the lead compound, has a higher vina score than Metformin, the standard medication.

TABLE 4.36: Docking result comparison

Ligand	**Vina score**	**Cavity volume**	**Centre (x, y, z)**	**Docking size (x, y, z)**
Metformin	-4.2	92	-8, -1, -6	17, 17, 17
Protocatechuic acid	-4.7	92	-8, -1, -6	17, 17, 17

Chapter 5

Conclusions and Recommendations

The objective of this study was to employ computational methods to identify potential compounds for the treatment of type 2 diabetes, with the goal of identifying efficient drug candidates for future use. Through comprehensive data mining of literature databases, a set of five ligands was selected for detailed investigation in this research. IRS1 was the preferred virtual screening protein. For docking investigations, CB Dock, an automated version of AutoDock Vina, was used. Ligplot Plus (version 1.4.5) was used to analyze protein-ligand interactions.

Protocatechuic Acid and P-Coumaric Acid, two notable phytocompounds, emerged as promising candidates after a thorough evaluation of their docking scores, physicochemical characteristics, and ADMET profiles. Based on their physicochemical and pharmacokinetic properties, their effectiveness as drugs was evaluated. Virtual screening revealed that protocatechuic acid was the lead compound because it had a higher affinity for binding to the IRS1 protein than metformin, a synthetic drug. This finding emphasizes the therapeutic potential of protocatechuic acid, which targets IRS1, in conditions influenced by insulin signaling pathways.

Due to its superior binding affinity for IRS1 in comparison to metformin, the virtual screening results identified protocatechuic acid as the lead compound. In

conditions like insulin resistance, this finding suggests that protocatechuic acid has the potential to be a therapeutic agent that targets IRS1-associated pathways.

Protocatechuic Acid outperforms metformin in terms of binding capacity and potential therapeutic efficacy in insulin signaling regulation. This work used online tools and computational methodologies to conduct a thorough evaluation of these substances, adding to the expanding body of knowledge about natural chemicals as alternatives or complements to synthetic medicines in diabetes care.

Metformin, a frequent type 2 diabetes therapy, activates AMP-activated protein kinase (AMPK), which lowers blood glucose levels and increases insulin sensitivity. However, its long-term use is limited due to the risk of gastrointestinal side effects and, in rare cases, lactic acidosis.With no reported adverse health effects, Protocatechuic Acid had potential IRS1 protein binding efficacy comparable to or superior to metformin. This wellbeing profile features its true capacity as a more secure other option or supplemental treatment for insulin opposition and other metabolic sicknesses.

The findings of this study are consistent with prior research indicating the usefulness of natural substances in addressing insulin resistance pathways. Protocatechuic Acid's superior IRS1 binding and activity demonstrate its promise for further research and development as a treatment agent for insulin-related diseases.

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