CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Insilico Approaches on Anti-Type 1 Diabetic Effects of *Moringa oleifera*

by

Ume Habiba

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To my beloved grandfather Abdul-Haq-Chugtai, uncle Zammurad Hussain, and especially to my father, (Tassadaq Hussain), whose constant courage, wisdom, and silent sacrifices have served as my beacon of light. My biggest inspiration has been your faith in me, even when I doubted myself. And I owe every action I've taken with confidence to my mother, whose love has always given me strength and comfort. This effort is a reflection of your prayers, unending support, and encouragement; it is not just mine.

I am grateful that you were the reason I never gave up.

CERTIFICATE OF APPROVAL

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Abstract

Type 1 diabetes (T1D) is a chronic autoimmune illness that develops when the body's immune system mistakenly attacks and destroys the insulin-producing β-cells in the pancreas. Although insulin therapy remains the foundation of treatment, it neither addresses the underlying causes of β-cell death nor offers long-term protection from its effects. Because of their possible therapeutic benefits and lower risk of adverse effects as compared to traditional medications, plant-based substances are attracting increasing scientific attention. The "miracle tree," *Moringa oleifera*, has been used in traditional medicine for thousands of years. It has a wide range of bioactive compounds that have been demonstrated to have antidiabetic, antioxidant, and anti-inflammatory properties. This work used an *insilico* method to thoroughly examine six key phytochemicals from *Moringa oleifera*: gallic acid, sitosterol, benzyl isothiocyanate (BITC), niazirin, niazimicin, and moringin. The human insulin (*INS*) protein, which is essential for controlling glucose, was targeted via molecular docking. To evaluate pharmacokinetic behavior and toxicity, ADMET profiling was employed. The common antidiabetic medication metformin served as the benchmark for comparison. Unlike earlier studies that focused solely on computer modeling or pharmacology, this work integrates molecular docking and ADMET predictions to provide a comprehensive evaluation of medication compatibility. The results indicate that, in the context of type 1 diabetes, benzyl isothiocyanate is a strong lead candidate for further investigation. It showed the best docking score (–8.0 kcal/mol) compared with other Moringa compounds and even outperformed metformin (–3.8 kcal/mol), while maintaining a safe ADMET profile. Overall, this work highlights the value of combining state-of-the-art in silico techniques with conventional botanical knowledge, while also confirming the therapeutic potential of *Moringa oleifera*. Because of its beneficial binding and safety profile, benzyl isothiocyanate is a promising natural agent that requires additional experimental model validation. Future studies should include both in vitro and in vivo trials to confirm its ability to sustain pancreatic β-cell activity, regulate glucose metabolism, and possibly function as an adjuvant or alternative treatment for Type 1 Diabetes.

Key words: ADMET profiling, Benzyl isothiocyanate, *In silico* analysis, Insulin protein, Moringa oleifera, Molecular docking, Type 1 Diabetes

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Abbreviations

ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
COPD	Chronic Obstructive Pulmonary Disease
DM	Diabetes mellitus
ER	endoplasmicreticulum
FPG	Fasting Plasma Glucose
GI	Glycemic Indexv
HbA1c	hemoglobin A1c
INS	Insulin Gene
LADA	Latent Autoimmune Diabetes in Adults
M.O	Moringa oleifera
MAFLD	Metabolic-Dysfunction Associated Fatty Liver Disease
MDA	Malondialdehyde
MODY	Maturity Onset Diabetes of the Young
OGTT	Oral Glucose Tolerance Test
PDB	Protein data bank
T1D	Type 1 Diabetes
T1D GRS2	Type 1 Diabetes Genetic Risk Score
T1DM	Type 1 Diabetes Mellitus
T2D	Type 2 Diabetes
TAC	Total Antioxidant Capacity

Chapter 1

Introduction

1.1 Background

Diabetes mellitus (DM), a disorder that changes how carbohydrates are metabolized, is certain to occur when the body's ability to make or react to insulin, which is necessary to keep blood sugar (glucose) levels within normal ranges, is diminished [1]. Type 1 and Type 2 *diabetes mellitus* are the two subtypes. Immune system activity is the primary cause of type 1 diabetes, resulting in β-cell death and total insulin insufficiency. Latent autoimmune diabetes in adults (LADA) is one kind of T1D that usually appears gradually as people age. Type 2 diabetes can range from a condition where insulin resistance is largely absent to one where insulin production is significantly reduced along with insulin resistance. High blood pressure, obesity, lipid metabolism problems, atherosclerosis, depression, obstructive sleep, chronic obstructive pulmonary disease (COPD), and metabolic-dysfunction associated fatty liver disease (MAFLD) were among the many medical conditions that are commonly associated with it. Endocrinopathies (such as Cushing syndrome, acromegaly, and pheochromocytoma), exocrine pancreatic diseases (such as pancreatitis, cystic fibrosis, pancreatic cancer, and post-pancreatic surgery, or "pancreatogenic diabetes"), drug-induced diabetes (such as that caused by glucocorticoids, neuroleptics, interferon-alpha, pentamidine, and streptozocin), infections (such as the mumps), and autoimmune-mediated diabetes are additional

specific types of diabetes. A specific kind of diabetes may also be linked to other hereditary diseases and genetic anomalies that impact β-cell function and Maturity Onset Diabetes of the Young [MODY] [2].

Diabetes is usually diagnosed using a range of clinical procedures that evaluate blood sugar levels: The Fasting Plasma Glucose (FPG) method is used to assess blood sugar after a person has fasted for at least eight hours. Two hours after consuming a glucose-rich beverage, blood sugar levels are measured using the Oral Glucose Tolerance Test (OGTT). The hemoglobin A1c (HbA1c) test indicates the normal blood sugar levels during the previous two to three months. Any time of day, a blood sugar level of 200 mg/dL or above, combined with symptoms of elevated blood sugar, such as increased thirst and frequent urine, which also point to diabetes, is known as random plasma glucose. An additional marker for tracking blood sugar control in diabetics is fructosamine, a glycated protein that, when combined with hemoglobin A1c (HbA1c), provides information on glucose management over a shorter time period, usually two to three weeks [3]. Globally, *diabetes mellitus* has become an epidemic, presenting a serious socioeconomic problem for developing countries. The prevalence of diabetes is expected to rise by 67% in developing nations between 2010 and 2030. Globally, 451 million individuals will have diabetes in 2017, and by 2045, that number is expected to increase to 693 million. Additionally, 49.7% of adult diabetics are thought to go untreated, with a large percentage living in low-income nations. Furthermore, it was projected that around 21.3 million live births would be affected by hyperglycemia of some kind during pregnancy, and approximately 374 million people will be reported to have impaired glucose tolerance [4].

Diabetes is predicted to affect 463 million people worldwide, with type 2 diabetes accounting for 90% of occurrences. According to an article in "The News," after China and India, Pakistan has the third-highest prevalence of diabetes globally. Diabetes would affect 11.77% of Pakistanis in 2016, 16.98% in 2018, and 17.1% in 2019. In 2022, 26.7% of Pakistani adults, or more than 33,000,000 people, are expected to have diabetes, according to the International Diabetes Federation. This figure is shockingly high and continues to rise annually. Furthermore, a

significant number of individuals are believed to remain undiagnosed, increasing both the actual prevalence and the risk of untreated cases [5].

The condition is caused by a number of risk factors. The primary risk factors for prediabetes and *diabetes mellitus* are genetics, environment, initial phase insulin release deficit, sedentary lifestyle, lack of physical exercise, smoking, alcohol intake, decreased β-cell sensitivity, hyperinsulinemia, and increased glucagon activity. These elements have a major role in insulin resistance or malfunction, which advances the course of the disease. About 90% of individuals develop type 2 diabetes, which is mostly linked to high body weight, according to WHO (2011). Common among overweight individuals, obstructive sleep apnea and sleep disturbances are important risk factors for insulin resistance and glucose sensitivity, which together cause prediabetes and then T2D. Diabetes is believed to be positively correlated with a diet low in fiber and high in glycemic index (GI) [6]. The increasing incidence of *diabetes mellitus* (DM) around the world has drawn attention from the World Health Organization (WHO), which has emphasized the importance of managing and fully comprehending its risk factors. Building on earlier studies, the WHO study for 2024 identifies a number of risk factors that contribute to the diabetes epidemic. Significant diabetes risk factors were summarized, with an emphasis on modifiable factors such as glycemic management, obesity, physical activity, dietary habits, age and so forth [7].

Type 1 diabetes is treated with insulin, protein, carbohydrate, and fat counts, regular blood sugar checks, a nutritious diet, regular exercise, and weight maintenance. The goal is to delay or prevent problems by keeping blood sugar levels as close to normal as possible. Before meals, blood sugar levels should typically be between 80 and 130 mg/dL (4.44 and 7.2 mmol/L), and two hours after meals, they should not exceed 180 mg/dL (10 mmol/L). People with type 1 diabetes must take insulin for the remainder of their lives, including short-acting (regular), rapid-acting, intermediate-acting, and long-acting insulin. Many persons with type 2 diabetes require insulin therapy or diabetes medicines, even though some can control their blood sugar levels with diet and exercise alone. A number of variables, such as blood sugar levels and other medical conditions, influence the

medication selection. To control blood sugar in a variety of ways, doctors may mix medications from various kinds [8]. The majority of individuals with type 1 diabetes receive four injections per day as part of "intensive" or "basal-bolus" insulin therapy. This method provides a great deal of flexibility with regard to meal time, portion amounts, and food types. Long-acting insulin at sleep and/or in the morning, nutritional insulin prior to each meal depending on carbohydrate content, and correctional insulin based on blood glucose readings prior to meals are all part of the standard treatment regimen[9].

Insulin-producing β-cells in the pancreatic islets of Langerhans are destroyed by T lymphocytes in type 1 diabetes, an autoimmune disease. Type 1 diabetes, the most severe kind, is sometimes referred to as "childhood," "early onset," or "juvenile-onset" diabetes. It can result in issues including blindness and renal failure. In humans, type 1 diabetes has only been consistently linked to and associated with two gene areas. The insulin gene (INS) region on chromosome 11 has a less impact than the major histocompatibility complex area on chromosome 6[10].

Despite weighing only 2 grams in humans, the pancreatic islets and the peptide hormones they release are essential for regular metabolic functions, especially the control of glucose through the creation and secretion of insulin by pancreatic beta cells. The translation of the INS gene product into preproinsulin initiates the protein synthesis process. The newly produced preproinsulin's signal peptide facilitates its enrollment to the endoplasmic reticulum (ER), where it is cotranslationally transported over the ER membrane and into the ER lumen. Proinsulin monomers are thought to fold and dimerize in the ER lumen; proinsulin comes into contact with zinc as it passes through the Golgi complex into immature secretory granules, which facilitates its assembly into hexamers. Insulin is eventually stored in mature secretory granules as a result of organelle remodeling and the proteolytic removal of the C-peptide and the dibasic residues that surround it [11].

Thirteen species of dicotyledonous trees that grow well in tropical and subtropical climates are part of the monogeneric Moringaceae family, which is made up of the single genus Moringa[12]. Before 2000 BC, ***Moringa oleifera*** was first identified as a medicinal plant in the sub-Himalayan areas of Bangladesh, India,

Pakistan, and Afghanistan. In addition to spreading westward to Egypt, the Horn of Africa, the Mediterranean, and ultimately the American West Indies, the Moringa tree also spread eastward to lower China, Southeast Asia, and the Philippines. The term ”Nebedaye,” which translates to ”never die,” is used in a number of African languages. It is also known as the ”Miracle Tree,” ”drumstick tree,” and ”horseradish tree.” Semiarid, tropical, and subtropical regions with sandy, dry soil are the primary growing environments for moringa. It is so resilient that it can tolerate severe drought and moderate frost. M. oleifera has long been used in Indian and African traditional medicine as an antioxidant, anticancer, anti-inflammatory, antidiabetic, and antibacterial agent. It is frequently considered a panacea, curing over 300 illnesses. Because its leaves are high in high-quality protein, its seeds are rich in lipids (mostly oleic acid, saturated palmitic acid, and stearic acid), and both the seeds and pods are high in calcium, potassium, salt, and iron, M. oleifera has a large nutritional value. Physicians, healers, dietitians, and community leaders will employ moringa extracts extensively to treat anemia and undernutrition, especially in young children and babies. Moringa has long been utilized to improve nutritional health, particularly when chronic diseases like diabetes, infections, or inflammation are present [13].

In cases of paralysis, the root portion possesses stimulant, anti-inflammatory, and anti-fertility properties. In addition to treating rheumatism, inflammations, articular aches, lower back or kidney pain, constipation, and relaxation, it also functions as a laxative, abortifacient, and cardiac/circulatory tonic. Leaf juice is believed to control glucose levels and reduce glandular edema. The leaves are also used to treat scurvy, catarrh, fevers, sore throats, bronchitis, eye and ear infections, and piles. They can also be applied as a poultice to wounds and massaged on the temples to relieve headaches. Stem bark is used to cure ulcers, eliminate tumors, stop splenic expansion and the growth of neck tuberculous glands, treat delirious patients, and treat eye disorders. The juice from the root bark has anti-tubercular qualities and is used to treat dental cavities and ears to ease pain. In addition to being used as an astringent and rubefacient to treat tooth cavities, gum can also be used to treat rheumatism, syphilis, fevers, intestinal issues, diarrhea, and asthma when coupled with sesame oil. An abortifacient is another usage for it. The flower

has great medical potential as a cholagogue, aphrodisiac, abortifacient, and stimulant. It is used to treat splenic enlargement, tumors, hysteria, muscular disorders, and inflammation. Additionally, it reduces the atherogenic index, cholesterol to phospholipid ratio, triglycerides, serum cholesterol, and phospholipid. It raises the excretion of fecal cholesterol and lowers the lipid profile of the liver, heart, and aorta in rabbits with hypercholesterolemia. Thiocarbamate and isothiocyanate glycosids, antihypertensive substances found in the acetate phase of the ethanolic extract of Moringa pods, have been demonstrated to have a protective effect by lowering liver lipid peroxides [14].

Approximately 40% of the global human insulin market is currently made up of pre-mixed insulin. Surprisingly little information is available regarding premixed mixes and comparisons with alternative delivery regimens, even though many patients will receive treatment with these insulin formulations. Increased dosage accuracy, effectiveness, and patient convenience will the primary benefits of utilizing a pre-mixed solution as opposed to self-mixed insulin. These advantages might boost adherence, which would improve the disease's long-term management. Many different types of people with T1D and T2D consume insulin combinations. Patients with diabetes who will old or adolescents also seem to benefit from premixed insulins [15].

The process of finding new drugs is difficult, time-consuming, and multidisciplinary. The use of *in-silico* chemistry and molecular modeling in computer-assisted drug creation has increased dramatically in recent years. Molecular biology, biochemistry, nanotechnology, and other fields use *insilico* drug design skills. Cost-effectiveness is the primary advantage of employing *in-silico* drug design in medical research and development. *In-silico* drug designs, Grid computing, window-based generic PBPK/PD modeling software, PKUDDS for structure-based drug design, APIS, Java, Perl, and Python, as well as softwwill libraries, are just a few of the many types of software that were utilized in this process. *In-silico* drug design visualization, homology, molecular dynamic, energy minimization molecular docking, QSAR, and other methods employ a variety of approaches. *In-silico* drug design has significant advantages for the whole drug development

process, from preclinical discovery to late-stage clinical development. It aids in selecting only a strong lead molecule during drug development and may help prevent late-stage clinical failures, thus saving a significant amount of money. A molecular modeling technique called docking looks at a molecule's preferred orientation to another when they combine to form a stable complex. Molecular docking indicates that a ligand has bound to its receptor or target protein. Molecular docking is used to find and enhance treatment possibilities by examining and modeling the molecular interactions between ligand and target macromolecules. Among the ligand conformations and orientations generated by molecular docking, the most appropriate ones will be selected. Several molecular docking technologies will be available, including CB Dock, CB Dock 2, Argus Dock, DOCK, FRED, eHITS, AutoDock, and FTDock. The affinities of ligands for binding to the active site of a receptor are rated using scoring procedures in molecular modeling. Compounds were dock into the active site and then be evaluated in virtual high throughput screening to determine which one has the best likelihood of forming a strong bond with the target macromolecule[16].

Secondary metabolites of plants, like as terpenes, coumarins, and phenolic compounds, are frequently held responsible for the effects of hypoglycemia. Although many secondary metabolites have shown hypoglycemic effects, little effort has been made to determine whether other substances, especially proteins, have a comparable function. It is demonstrated that M. oleifera's seed coat, seedless fruits, and leaves contain proteins that resemble insulin. M. oleifera seed coats and cotyledons undergo a process that uses acid-ethanol to extract insulin-like proteins from plant tissues, followed by acetone precipitation of the proteins. The precipitated components were collected by centrifugation and dialyzed extensively against distilled water using a 2-kDa cutoff cellulose membrane prior to freeze-drying [17].

1.2 Problem Statement

Type 1 diabetes is a chronic disease caused by the immune system destroying cells that produce insulin. Insulin therapy helps regulate blood sugar levels but does

not address the underlying cause of the condition. Conventional medications are often expensive, require rigorous daily use, and may have adverse reactions.

It is currently unknown how *Moringa oleifera*, a well-known medicinal plant with numerous beneficial properties, relates to Type 1 diabetes. The therapeutic potential of its natural ingredients has not yet been fully explored at the molecular level.

The problem this study tackles is the limited understanding of how natural compounds of *Moringa oleifera* work against diabetic targets and whether they're safe and effective. There's a need for computer-based (*insilico*) methods to predict their behavior and potential as alternative treatments.

1.3 Hypothesis

Moringa oleifera bioactive compounds exhibit potential anti-diabetic effects by interacting with key molecular targets involved in T1D pathogenesis.

The ability of *Moringa oleifera* compounds to resemble or improve insulin function will be determined by computer analysis (docking, ADMET tests).

1.4 Aim

The aim of the study was to investigate the potential therapeutic benefits of natural chemicals found in *Moringa oleifera* for the treatment of Type 1 Diabetes.

The investigation used computer-based *in silico* techniques to investigate the chemicals' interactions with important diabetes-related biological targets, evaluate their safety, and determine how well they might complement or enhance existing therapy options.

Their potential as natural, scientifically supported alternatives to treat the illness can be better understood with this method.

1.5 Objectives

1. To determine specific bioactive compound from *Moringa oleifera* useful for treatment of Type 1 Diabetes by using insilico approaches.

2. To use molecular docking techniques to study the strength and efficiency of these drugs' binding to significant protein targets associated with Type 1 Diabetes.

3. To assess the relative safety and effectiveness of *Moringa oleifera* compounds by contrasting their performance with that of a well-known anti-diabetic medication (like Metformin).

1.6 Scope

This study explores how natural compounds from *Moringa oleifera* may help manage Type 1 Diabetes using *insilico* approaches. It focuses on the mechanisms of action of these medications, their interactions with diabetes-related proteins, and their safety. This study will be useful to health industry and will cover SDG 3 (good health and well-being)

Chapter 2

Literature Review

2.1 *Diabetes mellitus*

A number of diseases related to metabolism are together referred to as *diabetes mellitus*, and they are all distinguished by consistently elevated blood sugar levels. This disease results from either a decrease in the efficacy of insulin or an insufficiency of insulin secretion, or sometimes from both. A condition affecting the metabolism of glucose, gestational diabetes is initially discovered during pregnancy. T1D, which is frequently caused by immunological causes, is characterized by the loss of β cells, resulting in a complete lack of insulin. Another type of *diabetes mellitus* is latent autoimmune diabetes in adults (LADA). Type 2 diabetes can range from merely insulin resistance with a relative lack of insulin to a significant secretory dysfunction with insulin resistance. It is frequently linked to other disorders, such as metabolic syndrome. Other distinct forms of diabetes that have known causes include endocrinopathies (like Cushing syndrome, acromegaly, and pheochromocytoma), diseases with pancreatic exocrine deficiency (like pancreatitis, hemochromatosis, and cystic fibrosis), drug or chemically induced conditions (like glucocorticoids, neuroleptics, interferon alpha, and pentamidine), and genetic defects that affect the function of β cells (like MODY types) or the action of insulin [18]. Figure 2.1 shows mechanism of *diabetes mellitus*.

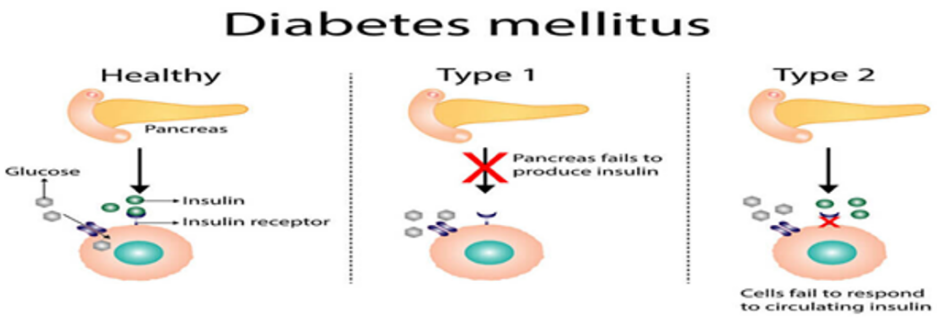


FIGURE 2.1: Types of *Diabetes mellitus* [19]

2.2 Type 1 *Diabetes mellitus* (T1D)

Type 1 *diabetes mellitus* (T1D) is a chronic autoimmune illness defined by high blood glucose levels, or hyperglycemia, caused by a lack of insulin due to the loss of pancreatic islet β-cells. One of the most common metabolic and endocrine conditions in children is type 1 diabetes. Patients with autoimmune type 1 *diabetes mellitus* (T1D) are classified as having T1D-associated autoantibodies and T1D-related autoimmunity, which is responsible for the death of β-cells in 70–90% of cases. Type 1b *diabetes mellitus*, also known as idiopathic T1D, has a strong hereditary basis, although the mechanism of β-cell loss is unknown in a smaller number of patients with no immune responses or autoantibodies [20].

Although it can be diagnosed at any age, type 1 diabetes is mostly a chronic disorder that affects children, with substantial incidences occurring around puberty and between the ages of 5 and 7. T1D is somewhat more common in men than women, in contrast to the majority of autoimmune disorders that mostly affect women. Seasonal changes and birth month have an impact on the prevalence of type 1 diabetes; more cases are diagnosed in the fall and winter, and people born in the spring are more likely to have the condition. Type 1 diabetes-related autoimmunity, such as the production of islet autoantibodies, frequently develops months or years before symptoms appear and also shows certain seasonal trends. These findings point to a possible environmental component that could initiate or intensify the pathogenic mechanisms of the illness. Type 1 diabetes prevalence and incidence vary greatly around the globe. It is most common in Sardinia, where

there are roughly 40 cases per 100,000 people annually, and Finland, where there are over 60 cases per 100,000 people annually. In contrast, there are about 0.1 instances per 100,000 people per year in nations like China, India, and Venezuela. Given the significant variations in disease rates even between adjacent regions of North America and Europe, the prevalence of type 1 diabetes worldwide poses an epidemiological conundrum. For example, although Estonia and Finland are fewer than 120 kilometers apart, Estonia's incidence is less than one-third of Finland's. Globally, the prevalence of type 1 diabetes has increased within the last few decades. There have been reported yearly rises of 2.4%, 2.6%, and 3.3% in Finland, Germany, and Norway, respectively. Sweden has recently seen a stabilization of incidence rates, although many other countries have seen varying rises. The global incidence may quadruple over the next ten years if current trends continue. Not all age groups have seen these increases equally; in Europe, children under the age of five have experienced the biggest increases. Although the exact causes of these regional differences and increasing incidence rates are still unknown, environmental factors are likely to be a contributing role. Genetic alterations or an increase in the number of children born to moms with type 1 diabetes are not the only factors contributing to the rapid increases in incidence. Finally, genetic predisposition seems to have a smaller role in type 1 diabetes development currently than it did in the past [21].

To prevent problems and improve long-term health outcomes, timely and accurate T1D screening is crucial. The importance of genetic risk scores in improving screening techniques is highlighted by recent studies. An enhanced Type 1 Diabetes Genetic Risk Score (T1D GRS2) was developed in one study to identify those who are more likely to develop T1D, allowing for earlier intervention and personalized treatment plans [22]. This development indicates a potential shift in screening protocols, enabling healthcare professionals to adopt preventive strategies based on genetic predispositions. Furthermore, a systematic review on celiac disease (CD) screening in T1D patients highlights the importance of routine screening at the time of diabetes diagnosis and at regular intervals thereafter. Considering the high occurrence of CD among T1D patients, incorporating CD screening into diabetes management is crucial for preventing complications and enhancing overall

care [23]. Despite these advancements, there are still notable gaps in screening practices, particularly concerning psychosocial factors influencing diabetes management.

Research shows that psychological well-being plays a significant role in adherence to treatment plans [24]. Therefore, there is an urgent need for screening tools that assess not only physical health indicators but also the psychosocial aspects of T1D management.

2.3 Treatment of T1D

The key component of treatment for type 1 diabetes is insulin therapy, which can be classified as intermediate-acting, long-acting, short-acting, or rapid-acting insulins.

2.3.1 Short-acting Insulin

Usually, 30 minutes after injection, short-acting insulin starts to work, peaks in around two to three hours, and lasts for six to eight hours. This kind of insulin is essential for controlling blood glucose levels during meals.

Because of its rapid start, patients can modify their schedules to accommodate meals; yet, due to its peak activity time, hypoglycemia is a concern [25].

2.3.2 Rapid-acting Insulin

Rapid-acting insulins, such as insulin lispro and insulin aspart, have an even quicker onset; they usually peak at one hour and last for two to four hours. This form of insulin works best for patients with severe post- meal hyperglycemia who have both type 1 and type 2 diabetes [26].

2.3.3 Intermediate-acting Insulin

Intermediate-acting insulin which normally has a duration of action of 10–16 hours, peaking between 4-6 hours after a 1-2 hour start. Because it lasts longer throughout the day, this kind of insulin is frequently utilized for basal insulin coverage [27].

2.3.4 Long-acting Insulin

Long-acting insulins that are made to release insulin gradually over a long period of time, usually up to 24 hours. These insulins don't have a noticeable peak in their effects and usually take 1-2 hours to start acting. Long-acting insulin is essential for controlling fasting blood glucose levels and for maintaining consistent insulin levels, which lessen glycemic variability [28].

Pharmacological treatments for type 2 diabetes are widely available and provide a range of choices for achieving glycemic control based on the particular needs of each patient. To control blood glucose levels and avoid problems, medication is required. The link between diabetes and cardiovascular health is highlighted by the fact that more recent anti hyperglycemic medications have been demonstrated to enhance glycemic management and lower cardiovascular events [29]. Another strategy for managing type 2 diabetes is to alter one's diet, particularly by eating plant-food polyphenols. These compounds may change how fats and carbs are metabolized and increase insulin sensitivity, which makes them a viable addition to traditional pharmaceutical treatments [30].

2.4 Global Prevalence and Burden

Diabetes mellitus is a chronic, severe illness that affects people worldwide, as well as their families and communities. It ranks among the top 10 causes of death for adults and is estimated to have killed four million people worldwide [31]. The International Diabetes Federation projects that in 2021, 537 million individuals

globally, or 10.5% of the total population, had diabetes, resulting in $966 billion in medical expenses [32]. It is alarming to see that the number of persons with *diabetes mellitus* is expected to increase to 643 million (11.3%) by 2030 and 783 million (12.2%) by 2045 [33]. Bangladesh, Brazil, Italy, Indonesia, Japan, Pakistan, India, China, and the United States are among the top ten nations in the globe for diabetes prevalence [34]. This health problem has thus turned into a worldwide emergency. Compared to high-income countries, the prevalence of *diabetes mellitus* is rising much more quickly in low- and middle-income countries (LMICs) [33]. It is crucial to keep in mind that LMICs account for over 80% of the global diabetic population, with the majority of diabetics living there [35]. Among the many socioeconomic problems that LMICs deal with include poor nutrition, deprivation, and inactivity. A recent review argues that precise, targeted data is badly needed to enable the establishment of successful programs meant to address these concerns [36]. Figure 2.2 also shows the increasing trend in the number of individuals (millions) with diabetes worldwide between the ages of 20 and 79.

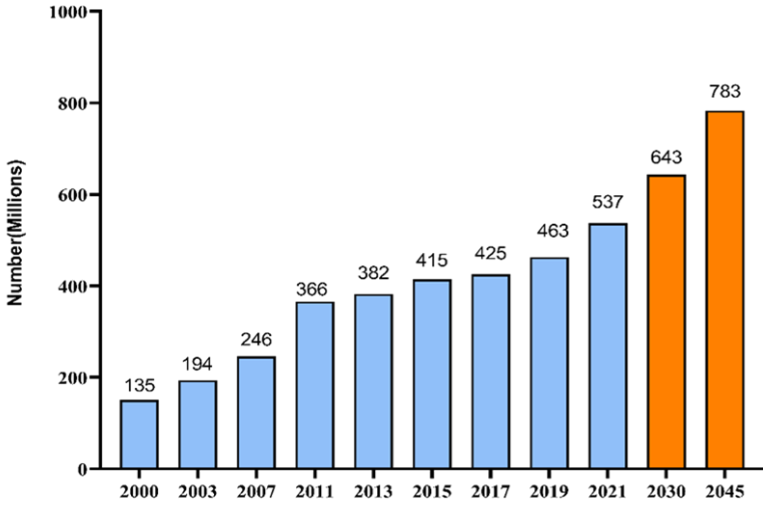


FIGURE 2.2: Global Prevalence of Diabetes [33]

2.5 Prevalence in Pakistan

Based on a sample of 49,418 people, the diabetes rate in Pakistan was determined to be 14.62%, suggesting a significant increase in the number of diabetes cases in

the nation. According to "The News" in Pakistan the number of people living with diabetes increased from 6.3 million in 2011 to 33 million in 2021 and approximately 36 million in 2024, with an additional one million as pre diabetic while Pakistan is set on way to having 62 million diabetics by 2045 [37]. The meta-analysis's research covered almost every geopolitical region in Pakistan, making it possible to evaluate regional differences in the prevalence of diabetes. Diabetes is common throughout the country, with Khyber Pakhtunkhwa having the lowest prevalence and Sindh province having the highest. Major risk factors for diabetes in Pakistanis include being older, having high blood pressure, being overweight, and having a family history of the condition [38]. Figure 2.3 shows prevalence of Diabetes in Pakistan according to international diabetes federation report in 2024.

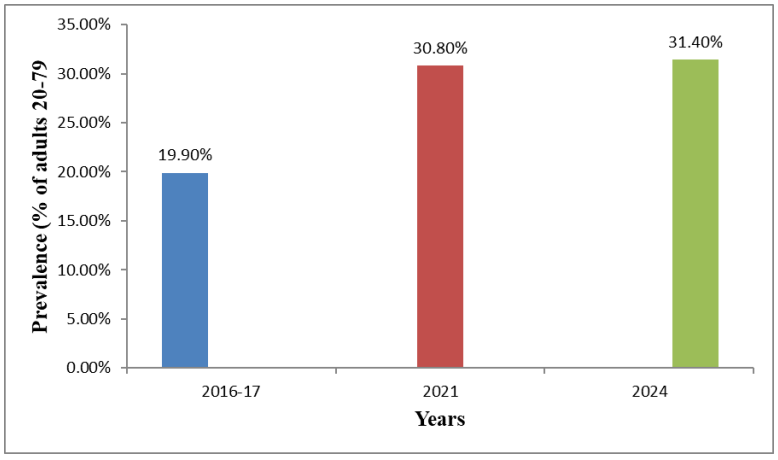


FIGURE 2.3: Prevalence of Diabetes in Pakistan [39]

2.6 *Human insulin gene (INS)*

The variable number of tandem repeats (VNTR) of the insulin gene is situated around 0.5 kb upstream of the gene. Class I (small, about 70% frequency in Caucasians, but over 90% in Japanese), class II (intermediate, rwill), or class III (big, around 30% frequency in Caucasians) are the classifications given to this polymorphic sequence. With a few small deviations, it is made up of a consensus sequence unit of 14–15 bp (ACAGGGGTCTGGGG). The insulin VNTR has been linked to T1D and is now known as the IDDM2 susceptibility locus. The shorter class I allele is associated with a vulnerability to type 1 diabetes, but the class III allele

is associated with resistance to the illness [40]. In the absence of glucose stimulation, ß-cells also exhibit low levels of INS transcription through a constitutive secretory path, resulting in to the sole release of proinsulin. It appears that the transcription factor Pur1 binding to the VNTR promoter region element mediates INS transcription in the steady state. The highly polymorphic VNTR locus's size and sequence changes may play a significant role in regulating INS transcription and diabetes risk [41].

2.6.1 Role of *INS* Gene in Diabetic and Healthy Individuals

The human insulin gene (*INS*), which has two introns and three exons, is found on chromosome 11p15.5. These exons encode the insulin molecule's A-chain, B-chain, C-chain, and signal peptide. It has long been thought that complex regulatory mechanisms involving the promoter of the gene and other transcription factors limit *INS* transcription to pancreatic ß-cells. When these transcription factors attach to particular promoter regions, more transcription co-activators are drawn in and a useful transcriptional complex is created that is only active in pancreatic ß-cells. In order to contribute to the early up-regulation of glucose-stimulated insulin production, glucose is known to increase *INS* translation and decrease *INS* mRNA degradation. Moreover, glucose stimulation increases the stability of *INS* mRNA by causing the polypyrimidine tract-binding protein (PTB) to bind to its 3'-untranslated region (UTR). By binding and stabilizing mRNAs encoding secretory granule proteins, activated cytosolic PTB increases the translation of those proteins. Glucose stimulation initiates the nucleocytoplasmic translocation of PTB. Through its various effects on a number of proteins linked to the transcription activation complex, including transcription factors essential for insulin secretion as well as for the growth and differentiation of the pancreas and ß-cells, glucose promotes *INS* transcription. Insulin expression in ß-cells is also influenced by post-transcriptional processes. Recently, it was discovered that the insulin gene message is alternatively spliced in the islets of the pancreas. The first 26 bp of intron 1, which is normally spliced out, is retained in this transcript. This

alternatively spliced transcript is expressed 50 times more frequently in human insulinomas than in healthy islets, although making up just 1% of the insulin mRNA in healthy islets.

Therefore, over-expression of this splice variant with improved translation efficiency may account for the retention of the high levels of insulin synthesis typical of insulinomas. This spliced version may also be upregulated in insulin-resistant individuals.

Although the alternative spliced variant has a considerably better translational efficiency, it nevertheless yields proinsulin protein with a normal amino acid sequence [41].

Defective proinsulin transport and elevated endoplasmic reticulum (ER) stress are believed to be the molecular mechanisms associated with disorders resembling MODY. Recessive mutations in the preproinsulin (*INS*) gene, a novel cause of neonatal diabetes, are the most frequent cause of isolated PND in children from closely related families. People who have both of their insulin alleles totally missing or inactive from birth have been known to have insulin-deficient diabetes [42]. The production of insulin by β-cells requires the expression of the *INS* gene.

Previous studies have shown that alterations in INS gene expression can significantly impact β-cell survival and function, which is important when T1D is present [43]. The transcriptomes of pancreatic α- and β-cells must be analyzed in order to identify components that may enhance insulin expression and encourage β-cell regeneration. This information could pave the way for customized therapies aimed at enhancing or reestablishing insulin production in people with T1D. Furthermore, T1D's heterogeneity makes it more difficult to comprehend its pathophysiology.

Genetic variables, such as polymorphisms in the *INS* gene, can cause variations in the onset, development, and response to therapy of disease. Because of this complexity, a more customized strategy that takes into account genetic backgrounds—particularly the function of the *INS* gene—is required rather than a one-size-fits-all strategy [44].

2.7 Plants Ethnobotanical Medicinal Importance

Ethnobotany is utilized in drug testing to pinpoint substances with pharmacological effects, resulting in the discovery of many beneficial compounds. Notable examples include aspirin, digoxin, quinine, and opium. Plants are rich in a variety of compounds, most of which fall into one of four biochemical categories: alkaloids, glycosides, terpenes, and polyphenols [45].

2.7.1 Drumstick Tree *Moringa oleifera*

A member of the monogenetic Moringaceae family, *Moringa oleifera* Lam. is a fast-growing tree that can grow to a height of five to ten meters. *M. oleifera* leaves are fluffy, light green, and between 30 and 60 cm long, with many tiny leaflets. The blooms have five uneven, creamy or white petals, be bisexual, and smell good. On hairy, slender pedicels, they bloom over the first six months after planting, usually from April to June, and eventually form a 12- to 30-cm anthemy. Seasonally chilly areas only get flowering once a year, but villas with constant seasonal temperatures and steady rainfall may see flowering twice or even all year. A tree can produce up to 1000 pods after three years; however fruit production is often minimal in the first few years. In hotter regions, the plants stay evergreen, but in subtropical villas, they become deciduous [46].Figure 2.4 shows the *Moringa oleifera* Plant, Leaves, Seeds and Flower.

FIGURE 2.4: *Moringa oleifera* Plant, Leaves, Seeds, Flower [47]

2.7.2 Phytochemical Structure

The health advantages of *M. oleifera* are mostly attributed to its rich profile of bioactive components, which include flavonoids, phenolics, glucosinolates, and carotenoids. The leaves are a valuable source of vitamins, proteins, and vital amino acids: research has shown that they contain significant levels of these nutrients [48]. Various accessions have various phytochemical compositions, which emphasize the value of genetic diversity in maximizing therapeutic potential through selective breeding [49]. Moringa leaves contain bioactive substances called flavonoids and phenolics that have been connected to a number of pharmacological actions, such as antibacterial and anti-inflammatory qualities [48]. Additionally, several investigations have revealed the existence of new substances such as Vicenin-2, demonstrating the medicinal potential of moringa in wound healing [50].

2.7.3 Nutritional Significance

The potential of moringa as a functional food additive has been investigated in recent research. Moringa has the potential to improve the nutritional value of a variety of food products because to its high concentration of bioactive chemicals [51]. Moringa may be added to diets to enhance general health and nutritional status because of the highlighted link between phenolic content and antioxidant capability. Moringa's function as a functional ingredient is further supported by the evaluation of its mineral bioaccessibility [52]. This emphasizes how crucial it is to comprehend the phytochemical profile in order to maximize its application in dietary applications and promote health benefits through increased nutritional value.

2.7.4 Traditional Uses

The moringa tree has many medicinal uses, including prevention and treatment. In many nations, traditional medicine makes use of its bark, sap, roots, leaves, seeds, oil, and flowers. In addition to being a blood builder and cleanser, it is a traditional

treatment for a variety of ailments, including diarrhea, cancer, stomach ulcers, skin conditions, blood sugar reduction, bone density enhancement, neurological disorders, diabetes, fatigue, increased lactation, hay fever, impotence, edema, cramps, hemorrhoids, headaches, and sore gums. To treat intestinal worms, the leaves were traditionally used as a poultice to the belly. Conjunctivitis is treated using an eye wash made from a leaf infusion. The soup made from drumstick leaves is excellent for preventing TB, bronchitis, and asthma naturally. The patient may choose to add lime juice, salt, and pepper to this drumstick leaf decoction, which is consumed as a soup. For both men and women, a decoction prepared with fresh drumstick blossoms and cow's milk is a great herbal remedy for functional infertility and sexual weakness. The powdered bark is used as medicine to increase the quality of semen and treat problems such male premature ejaculation. Premature ejaculation can be treated with a herbal remedy made from a decoction of bark powder and water with honey. Drumstick is a natural treatment for gastrointestinal issues. A glass of soft coconut water, one teaspoon of honey, and fresh leaf extract make a wonderful herbal cure for colitis, jaundice, diarrhea, cholera, and dysentery. A natural remedy for dysuria and high urine acidity is a fresh drumstick leaf extract combined with cucumber or carrot juice. Lime juice and drumstick leaf extract can be used to treat age spots, blackheads, and acne. It gives skin tone a natural glow when applied frequently [53].

2.8 Medicinal Uses

The leaves of the *M. oleifera* plant are most frequently used, but other parts of the plant have historically been used for different purposes. They were especially useful in traditional medicine and the nourishment of people and animals. Protein, calcium, beta-carotene, and antioxidants—all of which are commonly lacking in the populations of underdeveloped or impoverished countries—are all rich in the leaves. Food supplements made from moringa leaves are used. In traditional medicine, these leaves are used to treat a wide range of ailments, including genitourinary disorders, diabetes, typhoid fever, parasite infections, arthritis, swellings,

wounds, skin illnesses, and hypertension. They also boost the immune system (to treat HIV/AIDS-related disorders), promote lactation, and function as cardiac stimulants and contraceptives. The leaves can be eaten raw, dried, or extracted and used as an aqueous infusion. Likewise, the seeds will be utilized in traditional medication as well as human nourishment. To create beverages and infusions that can help with uterine illnesses, diabetes, anemia, hypertension, joint pain, toothache, hemorrhoids, impaired vision, and stomach issues (such as easing stomach discomfort, ulcers, and aiding in digestion), the bark is boiled in water and soaked in alcohol. Moringa seeds used a well-known process to remove impurities from water. Roots are steeped in water or alcohol and then cooked with other herbs as anthelmintic and antiparalytic drugs, sex enhancers, and toothache remedies. Last but not least, flowers are used to create aphrodisiacs to treat inflammation, hysteria, tumors, muscle diseases, and enlarged spleens [49].

2.8.1 Anti-cancer Activity

In research using mouse melanoma tumor models, alcoholic and hydromethanolic extracts of leaves and fruits showed a notable delay in tumor formation. Additionally, leaf extract showed antiproliferative effects on lung cells. Giving leaf extract to the chick chorioallantoic membrane produced a dose-dependent antiangiogenic impact, demonstrating its exceptional anticancer potential, according to studies on the conditions for cancer metastasis. Another study found that pod extract prevented male Institute of Cancer Research (ICR) mice from developing colon damage caused by azoxymethane and dextran sodium sulfate. In vitro, a root and leaf extract demonstrated cytotoxic effects on cisplatin-resistant ovarian cancer cells as well as colorectal, breast, and hepatocarcinoma cancer cells. While leaves extract shown strong antitumor and hepatoprotective properties, flower extract promoted cell proliferation in normal cells but not in cancer cells. These results point to MO's potential for regeneration in addition to its anticancer activities. Niazimicin, carbamates, thiocarbamate, nitrile glycosides, and other phytoconstituents like quercetin and kaempferol are what give this plant its anticancer properties [54].

2.8.2 Antimicrobial Activity

Because they contain pterygospermin and 4-a-L-rhamnosyloxy benzyl isothiocyanate, moringa roots have antibacterial qualities. The Moringa plant's pterygospermin has antibacterial properties against Helicobacter pylori, the bacteria that cause peptic ulcers. Benzyl isothiocyanates and other phenolic compounds found in M.O seeds are efficient against a variety of bacteria and fungi. Using the disc agar diffusion method, gram-positive bacteria (Staphylococcus aureus) and gram-negative bacteria (Escherichia coli, Salmonella typhi, Enterobacter aerogenes, Salmonella typhimurium, Shigella spp., and Pseudomonas aeruginosa) were the targets of an investigation into the antibacterial activity of chloroform and ethanol extracts from seeds and leaves. Bukar *et al.* [55] reported that the ethanol extract of seeds effectively inhibited three bacterial isolates: *S. aureus*, *E. coli*, and *S. typhi*. While the ethanol extract of leaves was efficient against *Enterobacter spp.*, *St. aureus*, *P. aeruginosa*, and E. coli, the chloroform extract of seeds was active against *S. typhimurium* and *E. coli*. *Bacillus cereus, Bacillus subtilis, Enterococcus faecalis, Micrococcus luteus, St. aureus, St. epidermidis, E. coli, Klebsiella pneumonia, P. aeruginosa, Serratia marcescens, S. paratyphi,* and *S. typhi* strains were all inhibited differently by different tissue extracts of M.O. Different tissue extracts inhibited different fungal strains, including *Aspergillus niger, Aspergillus paracitic, Candida albicans, Aspergillus flavus, Trichoderma harzanium, Alternata burnsi*, and *Fusarium oxysporum*. Bark, leaf, and seed extracts in ethanolic, methanolic, ethyl acetate, and water showed inhibitory effects on Aspergillus flavus mycelial growth [56].

2.9 Antioxidant Activity

The mitochondrial electron transport chain in cells is the main source of reactive oxygen species (ROS), which are linked to the development of a number of illnesses, including as diabetes, cancer, hyperlipidemia, cardiovascular disease, and dementia. According to earlier research, a number of plant extracts may be able to prevent or treat specific illnesses because of their antioxidant qualities. The

confirmation of antioxidant action is supported by these results. According to a 2009 study, *M. oleifera* leaves' polyphenols may be the cause of their antioxidant properties. In 2010, researchers discovered that *M. oleifera.* root, leaf, and stem bark extracts have strong antioxidant and radical scavenging qualities, with IC50 values of 8, 15, and 19 mg/ml, respectively. The same year, polyphenols from *M. oleifera* leaves, stems, and root barks were found to have antioxidant activity, which was linked to preventing cancer. It was also discovered that the volatile oil extracted from dehydrated *M. oleifera* leaves had antioxidant properties. Significantly, the chemical makeup of *M. oleifera* leaves is changed by various drying techniques, resulting in differences in the antioxidant and enzyme activity. Flavones and polysaccharides extracted from *M. oleifera.* leaves demonstrated the capacity to scavenge oxygen-free radicals using the DPPH assay. The methanolic extract of *M. oleifera* pods demonstrated a notable reduction in lipid peroxidation and an increase in proteins such as SOD, GSH, and CAT, in addition to the antioxidant qualities of *M. oleifera.* leaves [57].

2.10 Moringa olefira's Antidiabetic Properties

A number of the phytochemicals found in *M. oleifera* include antiviral, antibacterial, antioxidant, antitumor, immune-regulating, and anti-inflammatory qualities. They have also been demonstrated to help manage cholesterol, renal calculi, ulcers, diabetes, and hypertension. One of the main characteristics of diabetes, a group of dangerous and complex metabolic disorders, is hyperglycemia. Globally, diabetes is on the rise, which is extremely concerning. Because of this, diabetes prevention and treatment have received a lot of attention in our society, and the necessary actions will be taken to reduce this burden. An estimated 439 million persons globally are thought to have diabetes. Antibiotics were once used to treat diabetic mellitus, however in-vivo studies have shown that certain of them, such pentamide, are harmful to pancreatic β-cells. Streptozotocin is a broad-spectrum antibiotic that causes β-cells to vacuolate their mitochondria.

Additionally, it produces DNA strand breaks, dilates the endoplasmic reticulum, and lowers ATP levels in β cells. Numerous phytochemicals, such as those derived from *M. oleifera*, have demonstrated potential in the management of diabetes, particularly type 2. Aqueous, methanolic, and ethanolic extracts from various plant parts (leaves, pods, seeds, stems, and root barks) as well as dried leaf powder show great potential as an antidiabetic medication. Studies show that the residue from the aqueous extract of *M. oleifera* can treat type 1 diabetes and diabetes caused by streptozotocin. Studies employing a streptozotocin-induced diabetic mouse model demonstrate that *M. oleifera* leaf extract has significant antibacterial qualities and can help adult rats with *diabetes mellitus* regain the normal structure of their pancreatic β-cells. Some substances can damage the pancreas and make it more difficult for it to control elevated blood glucose levels, even while they act as antibiotics. Extracts from *M. oleifera* have been demonstrated to raise body temperature and reduce weight loss and metabolic parameters in rats with alloxan-induced hyperglycemia.

Additionally, *M. oleifera.* can lower postprandial hyperglycemia by inhibiting α-amylase and α-glucosidase activity. According to a kinetic investigation of glucose uptake in vivo, an aqueous extract of *M. oleifera* leaves may block glucose transporter activity, lower blood glucose levels, and decrease intestinal glucose absorption while also encouraging pancreatic β-cell regeneration. Quinine has been shown to have antidiabetic properties when it is isolated from different plants. *M. oleifera* leaf extracts can repair the pancreatic histological structure in alloxan-induced diabetic mice, and an alcoholic extract of the leaves shows that quercetin has a stronger antidiabetic effect than chlorogenic acid and moringinine. It is necessary to ascertain the ideal dosage of these substances, nevertheless. Furthermore, research has shown that low-dose aqueous extracts from *M. oleifera.* leaves have antidiabetic effects by inhibiting glucose transporter activity and reducing intestinal membrane glucose absorption. It was demonstrated in 2018 that *M. oleifera.* leaf powder decreased the amount of glucose absorbed by diabetic mice's stomach and skeletal muscle. These findings suggest that *M. oleifera.* leaves, either by themselves or in combination, may have antidiabetic effects. However, before evaluating it as a novel antidiabetic medication candidate, more research is required.

Because of its pharmacological potential, *M. oleifera*, which is abundant in proteins, phenols, vitamins, and minerals, has attracted a lot of attention. Research on animals has confirmed that *M. oleifera* has hypoglycemic effects. Giving *M. oleifera* leaf powder to patients with type II diabetes decreased their Low-density lipoprotein (LDL) and serum glucose levels, according to a clinical study aimed at the Indian population. To validate these results, nevertheless, a larger study with a more varied ethnic group is required [57].

Diabetes Types 1 and 2 can both be effectively treated with moringa. Rutin, quercetin-3-glycoside, and kaempferol glycosides are examples of polyphenols that can reduce blood sugar levels. Moringa's antidiabetic qualities are linked to its alkaloids, terpenoids, flavonoids, glycosides, and carotenoids. Rutin, quercetin-3-glycoside, and kaempferol glycosides are examples of polyphenols that also aid in the lowering of glucose. Low dosages of Moringa seed powder (50 or 100 mg/kg body weight) dramatically reduced the levels of IL-6 in the renal tissues of rats with diabetes induced by streptozotocin (STZ). Reactive oxygen species (ROS) are created when elevated blood glucose enters glycolysis in the beta cells' mitochondria, which can result in beta cell death, decreased insulin secretion, hyperglycemia, and eventually Type-2 diabetes. By neutralizing reactive oxygen radicals, moringa's antioxidant qualities help stop cell damage and apoptosis. Type I and insulin-resistant Type II diabetes can be treated with an aqueous extract of *M. oleifera* leaves, according to a study conducted on rats given STZ. In diabetic rats, the study found that Moringa leaf extract improved fasting blood glucose, gamma-glutamyl transferase, fasting plasma insulin, water intake, and weight loss. In rats, it also raised plasma albumin and decreased fasting plasma alanine aminotransferase and aspartate aminotransferase [56].

According to the studies in a diabetic rat model, Moringa seed powder dramatically improved biochemical indicators linked to diabetes. The findings highlighted Moringa's complex involvement in diabetes treatment by showing both a reduction in blood glucose levels and preventive benefits against diabetic nephropathy [58]. Additionally, Moringa pod methanol extracts had both antioxidant and antidiabetic effects in diabetic rats, indicating that oxidative stress reduction is a crucial

component of its therapeutic potential [59].

2.10.1 Mechanisms of Action

The antidiabetic effects of *M. oleifera* attributes to several mechanisms, including:

2.10.1.1 Enzyme Inhibition

M. oleifera phytochemicals slow down the absorption of glucose by inhibiting important enzymes involved in the digestion of carbohydrates, such as α-amylase and α-glucosidase [60–62].

2.10.1.2 Insulin Sensitization

M. oleifera extracts been demonstrated to increase insulin sensitivity through the upregulation of glucose transport proteins such as GLUT-4 and activating AMP-kinase [63, 64].

2.10.1.3 Antioxidant Effects

M. oleifera possesses potent antioxidant qualities that help lower oxidative stress and shield beta cells in the pancreas from harm [64, 65].

Chapter 3

Methodology

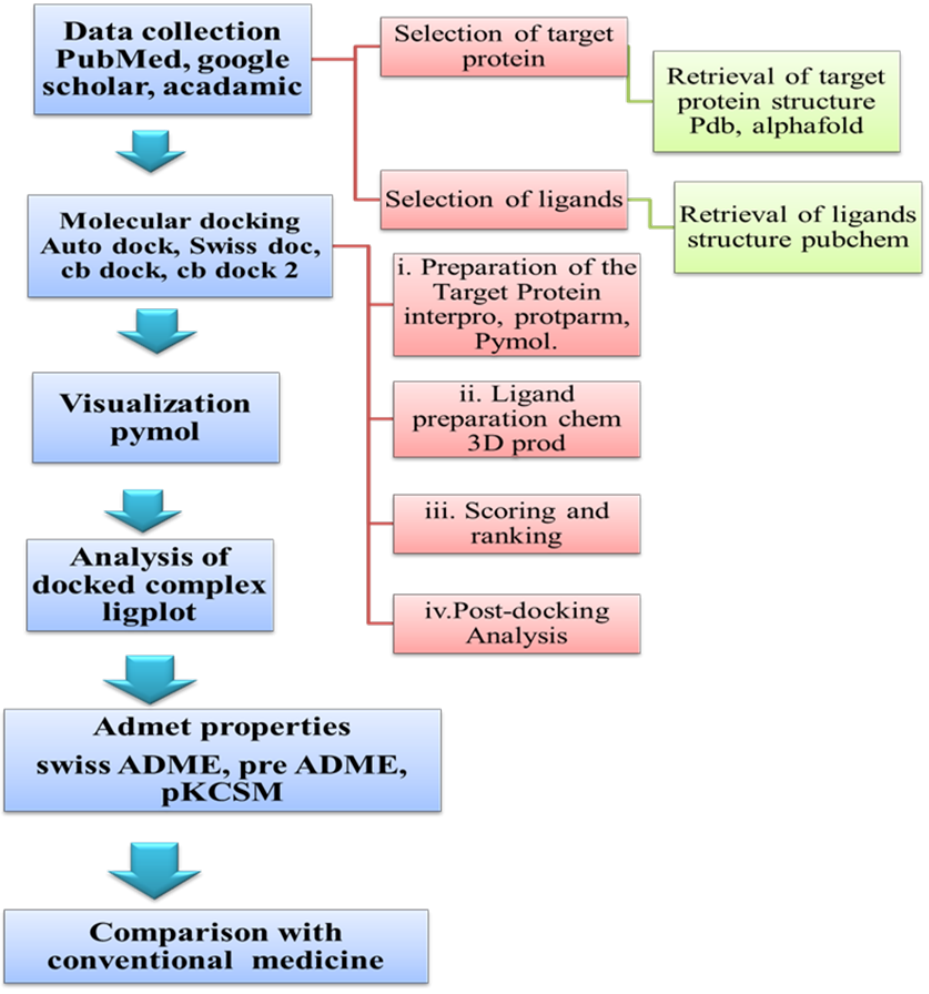


FIGURE 3.1: Methodology flowchart

3.1 Data Collection

Data collection and a literature analysis were crucial in examining *Moringa oleifera*'s anti-diabetic qualities since they provided a strong foundation for the development of *insilico* techniques. Examining previous research provided information on the different phytochemicals implicated in type 1 diabetes. A thorough study of the literature revealed a number of chemicals that showed promise in molecular docking experiments. In addition to emphasizing the chemical components, this method helped to clarify their structural and functional characteristics that are relevant to the treatment of diabetes.

3.2 Selection of Target Protein

The *INS* gene, which produces insulin, was selected as the study's target protein for a very specific and significant reason: insulin depletion is an essential component of type 1 diabetes. In individuals with type 1 diabetes, the immune system unintentionally targets and kills the pancreatic β-cells that produce insulin. Because of this, the body is unable to generate enough insulin to control blood sugar levels. As a result, the INS protein is a viable and significant target for any therapeutic intervention, and the insulin pathway is the primary cause of the illness [21].

3.3 Selection of Ligands

Benzyl isothiocyanate (BITC), gallic acid, sitosterol, niazirin, niazimicin, and moringin are the compounds from *Moringa oleifera* that were chosen because of their well-known natural medicinal qualities, particularly in relation to oxidative stress, diabetes, and inflammation. Previous research has demonstrated that these phytochemicals can lower blood sugar, lessen inflammation and damage from free radicals, and protect cells that produce insulin [65, 66]

3.4 Visualization of Structures

PyMOL is a robust cross-stage subatomic graphics tool available online at pymol.org that allows the investigation and visualization of proteins, small molecules, nucleic acids, electron densities, surfaces, and trajectories in three layers. It can create photos and films, alter molecules, and perform ray tracing, among other tasks.

Because PyMOL is built on Python and contains several plugin tools that expand its capability, it is an invaluable tool for drug targeting and design. In molecular biology and drug discovery, it is more practical and usable when used in conjunction with other applications, such MIX. After determining the protein structure, other protein-related components were eliminated using the open-source application PyMOL [67].

3.5 Molecular Docking

Molecular docking studies will act as a crucial *insilico* method to assess the potential antidiabetic effects of *Moringa Oleifera* by simulating interactions between the plant's phytochemicals and key protein targets linked to diabetes. This computational technique will enable predictions on how these bioactive compounds might inhibit enzymes critical in diabetic pathways. The current analysis will employ target proteins associated with type 1 diabetes, sourced from repositories like the protein data bank (PDB). Understanding protein activities and developing medication development depend on the ability to predict interactions between proteins and small molecules, which is critical for understanding a range of biological processes.

A powerful method for this is protein–ligand blind docking, which simultaneously determines a protein's binding locations and predicts a molecule's binding pose. The enormous number of protein structures identified by AlphaFold

or RoseTTAFold has recently led to a rise in the need for blind docking, opening up possibilities for the investigation of novel therapeutic targets. To investigate possible binding locations or ligand-binding poses, cutting-edge blind docking techniques like SwissDock, AutoDock, and CB dock, among others, have been extensively employed [68].

3.5.1 Preparation of the Target Protein

The first stage in molecular docking is obtaining the three-dimensional (3D) structure of the target protein, which is usually retrieved from databases like the Protein Data Bank (PDB).

Properly preparing this protein structure is vital and involves several steps: eliminating water molecules, incorporating hydrogen atoms, and assigning the correct charges to the protein [69, 70].

3.5.2 Ligand Preparation

After preparing the protein, the subsequent step is to prepare the ligand, where potential drug candidates are processed. This process includes generating the ligand's 3D structure, optimizing its geometry, and assigning charges.

Proper ligand preparation is crucial for accurate docking outcomes, as the ligand's geometric and electronic properties greatly affect binding interactions[71, 72].

3.5.3 Scoring and Ranking

The next important step is to score and rank the ligand-protein interactions. This is done by assessing binding energies and other interaction metrics to identify the most favorable binding conformations. Effective scoring systems are essential for differentiating between high-affinity and low-affinity interactions among various ligands [73].

3.5.4 Post-Docking Analysis

Post-docking analysis involves a thorough evaluation of the docking results. This includes visualizing binding poses and analyzing the interactions between the ligand and protein. This step is crucial for understanding the dynamic nature of the ligand-protein interactions and ensuring that the predicted binding modes are realistic [16, 74].

3.6 Lipinski's Rule

Early on in the drug design process, medicinal chemists have found great success using Lipinski's Rule of Five. According to the rule, compounds are more likely to have favorable pharmacokinetic properties if they have no more than one violation of the following criteria:

a) No more than five hydrogen bond donors.

b) No more than ten hydrogen bond acceptors.

c) A molecular weight less than 500 Da.

d) A calculated log P (lipophilicity) of less than 5.

e) The number of rotatory bonds should be less than 5.

The usefulness of Ro5 as an initial filter for locating promising drug candidates based on their ADMET profiles has been supported by numerous research [76].

3.7 ADMET Properties

Predicting pharmacokinetic properties such as Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) through computational methods is a vital part

of contemporary drug discovery processes. For natural substances like the bioactive compounds found in *M.O*, understanding ADMET features through computational analysis can speed up lead optimization, cut costs, and lower the likelihood of failure during preclinical stages. The aim is to assess the drug-likeness, pharmacokinetic profile, and toxicity risks of specific phytochemicals from *M.O* using *in silico* ADMET prediction tools, thereby evaluating their potential as anti-diabetic agents for Type 1 *Diabetes mellitus* (T1DM). Precise ADMET profiling can forecast oral bioavailability, permeability, and systemic toxicity of potential compounds. Natural products often encounter setbacks due to inadequate pharmacokinetics; thus, early evaluation using computational tools is crucial for streamlining the drug development process [77].

3.8 Analysis of Docked Complex

To interpret the docking results, the interactions between the protein and the ligand's active pockets are calculated. Hydrophobic and hydrogen bonding interactions are the two kinds of interactions that are examined. LigPlot+ (version 1.4.5), a program that automatically creates schematic diagrams of protein-ligand interactions based on the supplied PDB files, was used to study these interactions. Understanding the type and intensity of the interactions between the ligand and the protein is made easier by this visual portrayal [78].

3.9 Lead Compound Identification

After a thorough examination of the protein and its interactions, the compound with the highest docking score and toxicity tests is identify as the lead compound.

Chapter 4

Results

The results obtained from our methodological procedures are compiled in this chapter. The inputs were the three-dimensional structures of the ligands and proteins. Following domain prediction and physicochemical parameter analysis, the proteins were docked with the selected ligands, whose energies had already been lowered. The ADME/T characteristics and Lipinski's rule were used to predict the compounds' drug-like properties. By contrasting the found chemical's characteristics with those of already-approved drug, its validity was further confirmed. Each of these processes is explained in depth in the headings that follow.

4.1 Structure Modeling

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

4.1.1 Sequence Retrieval

Finding a protein's main sequence is an important first step. Typically, this procedure entails using specific identifiers or phrases to search for the protein of interest in reputable databases like UniProt, Alpha fold or the Protein Data Bank (PDB).

The sequence of *INS* 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. The sequence of *INS* in fasta format is shown in figure 4.1.

```
>AF_AFP01308F1_1|Chain A|Insulin|Homo sapiens (9606)
MALWMRLLPLLALLALWGPDPAAAFVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAEDLQVG
QVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLYQLENYCN
```

FIGURE 4.1: Sequence Retrieval

4.1.2 Physiochemical Characteristics of Proteins

Physiochemical characteristics of proteins are determined by examining elements such as molecular weight, instability index, isoelectric point (pI), and amino acid composition. This procedure requires the use of Protparam, an online tool provided by the ExPASy Bioinformatics Resource Portal.

I obtained a comprehensive analysis of a protein's physiochemical characteristics by inputting its amino acid sequence into Protparam. This involves calculating attributes like GRAVY (grand average hydropathicity), theoretical pI, molecular weight, and amino acid concentration.

A protein's basic nature is indicated by a computed pI greater than 7, whereas an acidic nature is indicated by a PI less than 7. Light absorption is represented by the extinction coefficient. Protein stability is indicated by an instability index of less than 40, whereas protein instability is indicated by an index greater than 40.

These measurements offer crucial information about the structural and functional characteristics of proteins, which facilitates their description and comprehension of their roles in biological processes. The physiochemical properties like number are amino acids, molecular weight and instability index is shown in Table 4.1.

The aliphatic makeup of a protein is measured by the aliphatic index. The heat stability of the protein is indicated by a high aliphatic index. Protein residues'

molecular weight indicates whether they are positively or negatively charged. Better interactions with water molecules are indicated by a low GRAVY.

Table 4.1: Physicochemical properties of target proteins

Protein	**Properties**	**Score**
INS	Number of amino acid	110
	Molecular weight	11980.91
	Theoretical pI	5.22
	-ive residues	10
	+ive residues	7
	Total atom	1680
	Ext. Co1	17335
	Ext. Co2	16960
	Instability index	40.33
	Aliphatic index	102.91
	Grand average of hydropathicity	0.193

4.1.3 Predicting Protein 3D Structures

Using the Protein Data Bank (PDB), the *INS* protein's 3D structure was predicted. Clarifying *INS* structural properties was the main goal in order to comprehend its function in insulin signaling networks and metabolic diseases.

In this study, the Protein Data Bank (PDB) and AlphaFold resource were used to obtain the 3D structure of the *INS* (insulin) protein. The PDB is a reputable database that offers experimentally confirmed protein structures, frequently obtained using methods like NMR spectroscopy or X-ray crystallography. In order to facilitate molecular docking research, pertinent structural information on insulin was obtained from the PDB.The projected three-dimensional structure of the insulin protein, identified by the AlphaFold ID AF_AFP01308F1, was also obtained from the AlphaFold Protein Structure Database.

DeepMind's AlphaFold predicts protein folding with extraordinary accuracy using deep learning and artificial intelligence, particularly for proteins without high-resolution experimental structures.During molecular docking, this predicted structure was utilized to model interactions with bioactive chemicals to find possible binding sites.After the water molecules and any ligands were eliminated, the protein structures were prepared in PyMOL. Polar hydrogens that were absent were then added to the structures. After that, the energy reduction procedure was carried out to produce stable conformations and prevent structural overlaps.

Lastly, PDB format was used to preserve the modified structures. Following the above-described preparation procedures, the optimum conformations of the refined structures are shown in the figure 4.2.

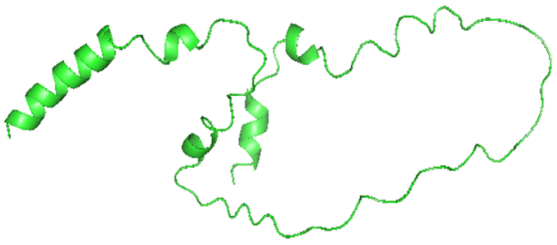


FIGURE 4.2: 3D Structure of Insulin Protein

4.1.4 Functional Domain Identification

The primary regions of the insulin protein that are in charge of its biological action are seen in its functional domains. Insulin-like domains, which are frequently present in both insulin and its cousins, such as insulin-like growth factors (IGFs), make up a sizable portion of the insulin sequence. The hormone's capacity to attach to its receptor and initiate physiological processes depends on these regions. The insulin domain, the active core located in the center of the protein, is directly in charge of attaching to the insulin receptor and performing insulin's principal job of controlling blood sugar levels. IGF-1, a hormone that also affects development and metabolism, overlaps this, demonstrating the structural and functional similarities between insulin and IGF-1. Together, these domains demonstrate how

insulin is carefully controlled to support essential processes including glucose uptake and cellular energy balance. The main active component of the hormone, the insulin domain shown in Figure 4.3 in purple, is necessary for interaction with the insulin receptor.

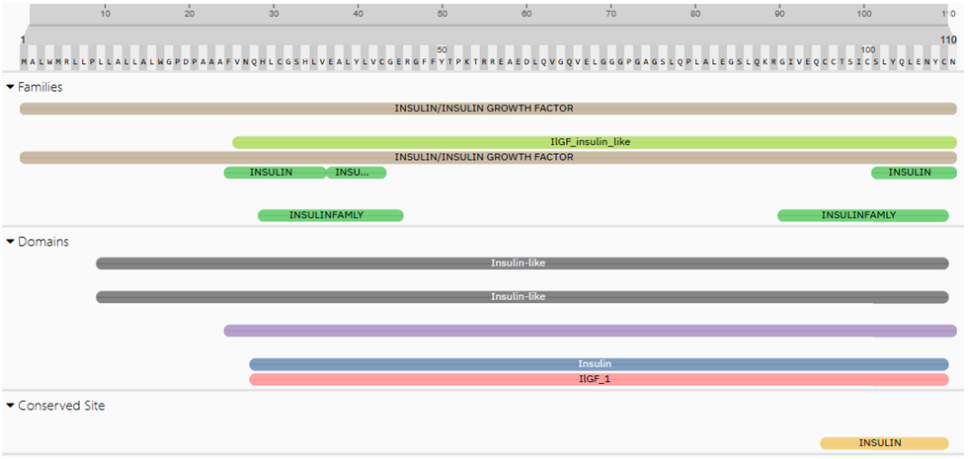


FIGURE 4.3: Functional Domains of protein.

4.2 Ligand Selection

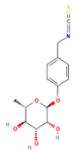
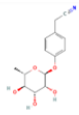
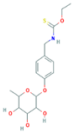
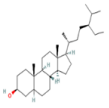
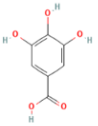
A vast array of protein-ligand interactions that are essential for structural biology and drug development can be found in the Protein Data Bank (PDB). Strong binding affinities, pertinent chemical classes, and high-resolution structures are the main criteria used to select ligands. The generation of stable complexes is the outcome of conformational selection, a critical process in which a ligand binds and stabilizes a certain protein conformer, increasing the population of that conformer. A comprehensive approach to determining and improving efficient protein-ligand interactions is achieved by optimizing ligand selection using protein dynamics, ligand efficiency, structure-activity relationship (SAR) data, computational predictions, and functional experiments.

The chemical information database PubChem, pubchem.ncbi.nlm.nih.gov, was searched for ligands. The Protein Data Bank (PDB) offers worldwide access to data on interactions between proteins and ligands, including 3D ligand structures

that may be downloaded from PubChem in SDF format. Chem 3D software (version 12.0.2) is used to minimize energy after the ligands have been chosen. The Lipinski rule of five should be followed while preparing ligands for docking: molecular weight 500, log P 5, hydrogen bond donors 5, and hydrogen bond acceptors 10. By avoiding unstable ligands, this step improves the stability and dependability of docking data from generating faulty Vina scores. The Lipinski rule for orally active compounds was followed by the selection of Moringin, Niazirin, Niazimicin, Benzyl isothiocyanate, Sitosterol and gallic acid as ligands in the current investigation.

Orally active chemicals must adhere to these guidelines. The drug's effectiveness depends on how it is administered. Ligands with their structures and molecular formula are shown in Table 4.2.

TABLE 4.2: Structure of Ligands

Sr.	**Ligand Name**	**Molecular Formula**	**Molecular Weight**	**Structures**
1	Moringin	$C_{14}H_{17}NO_5S$	311.36 g/mol	
2	Niazirin	$C_{14}H_{17}NO_5$	279.29 g/mol	
3	Niazimicin	$C_{16}H_{23}NO_6S$	357.4 g/mol	
4	Benzyl isothiocyanate	C_8H_7NS	149.21 g/mol	
5	Sitosterol	$C_{29}H_{52}O$	416.7 g/mol	
6	Gallic acid	$C_7H_6O_5$	170.12 g/mol	

4.3 Active Site Identification

By forecasting accessible binding pockets and supplying surface area and volume data, the CASTp algorithm finds active protein sites. A computer technique for determining and describing the topography of protein structures is called CASTp (Computed Atlas of Surface Topography of Proteins). In order to comprehend protein-ligand interactions, enzyme active sites, and other functional areas, it includes comprehensive information about the pockets and cavities on the protein surface. CASTp calculates different geometric parameters including area, volume, and the number of accessible surface points while analyzing protein structures to find surface features. Figure 4.4 represents the available pockets of the *INS* protein for the ligand.

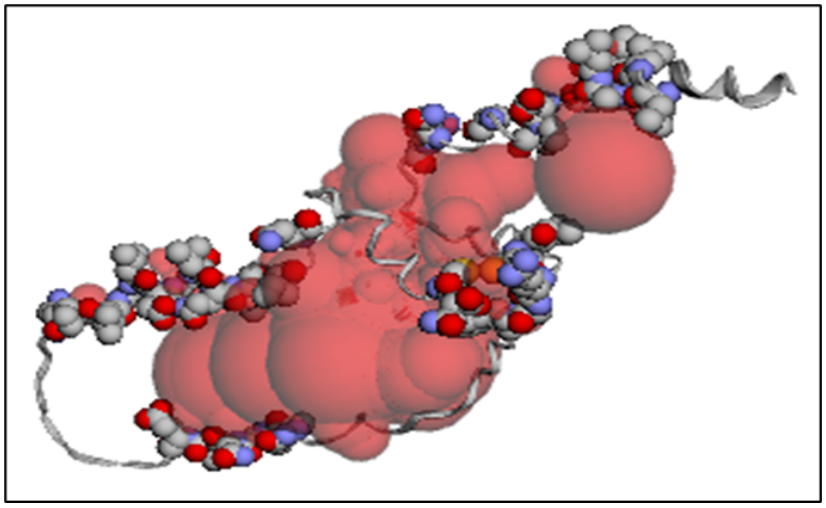


FIGURE 4.4: Structure of *INS* protein representing available pockets for ligands.

The protein's accessible binding pocket is indicated by the red tint. A region where ligands can bind is called the binding pocket. The sizes, quantity and volumes of the pockets are shown in the Table 4.3.

TABLE 4.3: Area and volume of binding pockets of *INS*

Pocket ID	**Arear (SA)(A^2)**	**Volume (SA)(A^3)**
1	1868.02	5616.346
2	92.84	384.247
3	18.044	61.09

continued on next page

Table 4.3 continued from previous page

Pocket ID	Arear (SA)(A^2)	Volume (SA)(A^3)
4	28.017	8.721
6	19.502	4.082
7	5.063	2.608
8	3.815	0.769
9	0.757	0.091
10	3.32	-0.578

The binding pocket IDs, area, and volume of accessory gene regulator protein *INS* are displayed in table 4.3 above. It shows that 10 pockets are available for protein *INS*. The largest binding pocket has a volume of 5616.346 and a surface area of 1868.020.The smallest pocket has a volume of -0.578 and a surface area of 3.320.

4.4 Ligand and Target Protein Interaction

To interpret docking results, the interaction between the protein and the ligand's active pockets was estimated. Hydrogen bonding and hydrophobic bonding interactions were the two types of interactions that were examined. Protein-ligand interactions were investigated using Ligplot Plus (version v.1.4.5). The interaction between target proteins and active confirmation ligands was determined using Ligplot. We thoroughly examined each molecule's stored conformations in the ligand-receptor combination.

4.5 ADMET Properties of Ligands

Artificial availability and verbal bioavailability are assessed using Lipinski's five-drug law. In a second study, the online tool pkCSM was used to compute the ADMET properties of ligands as a pharmacokinetic metric. Pharmacodynamics and pharmacokinetics are the two main terminologies used in pharmacology.

The process by which a drug enters tissues from the bloodstream is known as absorption in pharmacology. The chemical characteristics of the medicine and its surroundings affect the rate and degree of its absorption. Drugs must pass through endothelial or epithelial cellular barriers in order to be absorbed. It takes energy to go against a concentration gradient, and only a few number of medications can actively penetrate these barriers. Medication frequently diffuses passively through cell membranes from regions of high concentration to regions of low concentration. Energy is not needed for this procedure, however the drug's size and solubility have an impact.

Medication frequently diffuses passively through cell membranes from regions of high concentration to regions of low concentration. Energy is not needed for this procedure, however the drug's size and solubility have an impact.

The transfer of medications from one area of the body to another is referred to as distribution in pharmacology. The volume of distribution in humans (VDss, expressed in log L/kg) is one of the four ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics. The human unbound fraction (Fu), blood-brain barrier permeability (log BB), and central nervous system permeability (log PS) are additional significant variables.

4.5.1 Moringin

Moringin is a naturally occurring substance that is somewhat absorbed in the gut, while efflux proteins like P-gp may push some of it out. It lowers the likelihood of CNS adverse effects since, once in the body, it primarily remains in the blood and does not enter the brain. Since liver enzyme issues are unlikely to arise, it can be taken with other medications without raising too many concerns about potential interactions.

It is non-toxic and clears steadily; it doesn't appear to be causing liver toxicity, heart issues, or genetic damage. Overall, niazimicin is a safe and promising natural substance; nevertheless, several formulation changes could improve its absorption and increase its bioavailability.

4.5.1.1 Absorption

Moringin solubility in water is -2.64 log mol/L which indicates the degree to which it dissolves in water. It may have trouble dissolving in stomach juices if the number is negative, which indicates that it is moderately soluble. The permeability of Caco-2 is 0.376 log Papp. The gut wall is mimicked by Caco-2 cells. Moringin may have trouble crossing the intestinal lining if this value is low. It implies a limited capacity for absorption.58.88% of the absorption occurs in the stomach. The bloodstream could absorb more than half of the moringin taken orally. It's manageable, not too high, but moderate. Permeability of Skin -3.048 log Kp means it is unlikely that moringin will be absorbed through the skin. Therefore, oral use is more appropriate than patch or cream use. The P-gp, a gut pump that pushes medications back into the gut and decreases absorption, recognizes moringin. Therefore, rather of being absorbed, some moringin may be pushed out.No P-gp Inhibitor since moringin does not affect P-gp, it will not impede the absorption of other medications. The absorption properties are shown in Table 4.4.

Table 4.4: Absorption properties of Moringin

Property	**Model name**	**Predicted value**
Absorption	Water solubility	-2.64
	Caco2 permeability	0.376
	Intestinal adsorption (human)	58.884
	Skin permeability	-3.048
	P-glycoprotein substrate	Yes
	P-glycoprotein I inhibitor	No
	P-glycoprotein II inhibitor	No

4.5.1.2 Distribution

The volume of distribution VDss value is -0.216 log L/kg which implies a reasonable spread of tissue. It is likely that moringin does not spread widely into tissues, instead remaining in the circulation. Unbound Fraction (0.443 Fu) in the blood, about 44% of moringin is still free and active. Proteins bind the remainder. This

free fraction is respectable and permits some activity. Permeability of the Blood-Brain Barrier (BBB) is -0.837 because moringin is unlikely to enter the brain, it can help prevent undesirable central nervous system (CNS) side effects. Permeability of CNS is -3.031 moringin's limited brain accessibility lowers the possibility of CNS-related harm, supporting the aforementioned. The distribution properties are shown in table 4.5.

TABLE 4.5: Distribution property of Moringin

Property	**Model name**	**Predicted value**
Distribution	VDss(human)	-0.216
	Fraction unbound	0.443
	BBB permeability	-0.837
	CNS permeability	-3.031

4.5.1.3 Metabolism

Moringin does not inhibit or get metabolized by key CYP enzymes (such as CYP3A4, 2D6, 1A2, etc.); this reduces the likelihood of drug interactions with other medications that the liver processes.This indicates that moringin is likely not primarily metabolized by the usual liver enzymes but rather by alternative mechanisms (such as conjugation like glucuronidation).The distribution properties are shown in Table 4.6.

TABLE 4.6: Metabolism property of Moringin

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

4.5.1.4 Excretion

Clearance Total is 0.283 mL/min/kg means this is how quickly the body eliminates moringin. The number indicates a reasonable clearance, neither too quick nor too slow. Since moringin is not eliminated by the kidney transporter OCT2, it most likely excretes through alternative routes. The excretion properties are shown in Table 4.7.

TABLE 4.7: Excretion property of Moringin

Property	**Model name**	**Predicted value**
Excretion	Total clearance	0.283
	Renal OCT2 substrate	No

4.5.1.5 Toxicity

Moringin is not mutagenic.No hERG Inhibitor It is unlikely to result in problems with cardiac rhythm, which is a major worry with medications. Maximum Tolerable dose is 0.466 log mg/kg/day indicating that moderate dosages of moringin can be tolerated without causing significant harm. Acute Toxicity in Oral Rats is LD50: 2.136 mol/kg means moringin is not extremely harmful in single doses because the fatal dose is somewhat high. When administered over an extended period of time, oral rat chronic toxicity (LOAEL: 2.089 log mg/kg_bw/day) indicates a minimal toxicity risk. Moringin poses no significant risk of liver injury. Not likely to irritate or trigger allergic reactions. The toxicity of moringin is shown in Table 4.8.

TABLE 4.8: Toxicity property of Moringin

Property	**Model name**	**Predicted value**
Toxicity	AMES toxicity	No
	Max. tolerated dose (human)	0.466
	hERG I inhibitor	No
	hERG II inhibitor	No
	Oral Rat Acute Toxicity (LD50)	2.136
	Oral Rat Chronic Toxicity (LOAEL)	2.089
	Hepatotoxicity	Yes

Table 4.8 continued from previous page

Property	Model name	Predicted value
	Skin Sensitation	No
	T.Pyriformis toxicity	0.277
	Minnow toxicity	2.744

4.5.2 Niazirin

A potential natural substance with a well-balanced safety and absorption profile is niazirin. Although it is reasonably absorbed from the gut and has moderate water solubility, its distribution is restricted, primarily remaining in the bloodstream with minimal penetration into the brain. It has a modest risk of drug interactions because it doesn't affect the main liver enzymes. Niazirin is excellent since it is non-toxic to the heart and liver and is eliminated at a healthy rate. Although its mild environmental impact on aquatic systems should be taken into consideration, its overall safety profile is positive.

4.5.2.1 Absorption

Solubility in Water is -2.237 log mol/L although niazirin is not very soluble in water; it is more soluble than several natural substances, such as sitosterol. This indicates that it can partially dissolve in the digestive tract's aqueous environment, facilitating absorption. Permeability of Caco-2 is 0.48 log Papp because niazirin has a modest ability to pass through intestinal walls, it can reach the bloodstream at a respectable but not very high rate. The intestinal absorption rate is 58.333%. The intestinal tract absorbs about 58% of niazirin, which is a reasonable absorption rate. It is inferior to highly permeable compounds but superior to sitosterol. Permeability of Skin is -2.965 log Kp. Topical delivery would not be the best option because niazirin is unlikely to permeate the skin well. A portion of the absorbed Niazirin may be pumped out of cells, lowering its effective concentration in the body. Niazirin is recognized by P-gp transporters. P-gp Inhibitor I and II are both negative. Niazirin won't interfere with the absorption or clearance of

other medications because it doesn't disrupt P-gp transporters. The Absorption properties of Niazirin is shown in Table 4.9.

TABLE 4.9: Absorption properties of Niazirin

Property	**Model name**	**Predicted value**
Absorption	Water solubility	-2.237
	Caco2 permeability	0.48
	Intestinal adsorption (human)	58.333
	Skin permeability	-2.965
	P-glycoprotein substrate	Yes
	P-glycoprotein I inhibitor	No
	P-glycoprotein II inhibitor	No

4.5.2.2 Distribution

The Human VDss value is -0.211 log L/kg. Niazirin rarely penetrates deeper tissues, preferring to remain primarily in the blood plasma. This could reduce its ability to accomplish some goals. Unbound Fraction 0.419 Fu reflects that Niazirin can interact with bodily tissues or targets because about 42% of it is still free in the bloodstream. Most likely, the remainder is protein-bound.

Permeability of the BBB (-0.65 log BB) Niazirin is unlikely to directly affect brain tissues because of its limited blood-brain barrier crossing capabilities. Permeability of the CNS is -3.016 log PS. Its utility for brain-targeted therapy is also limited by its extremely low capacity to penetrate the central nervous system. The distribution property of niazirin is shown in Table 4.10.

TABLE 4.10: Distribution property of Niazirin

Property	**Model name**	**Predicted value**
Distribution	VDss(human)	-0.211
	Fraction unbound	0.404
	BBB permeability	-0.65
	CNS permeability	-3.016

4.5.2.3 Metabolism

CYP3A4, CYP1A2, CYP2D6, CYP2C9, and CYP2C19 are the main liver enzymes that niazirin neither inhibits nor substrates. The good news is that Niazirin is unlikely to disrupt drug metabolism, which lowers the possibility of drug interactions. The metabolism property of niazirin is shown in Table 4.11.

TABLE 4.11: Metabolism property of Niazirin

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

4.5.2.4 Excretion

The body eliminates niazirin at a moderate rate of 1.165 mL/min/kg. It's neither too slow to run the risk of accumulation nor too rapid, so it can remain in the system long enough to be useful. Niazirin probably exits the body through the liver or bile because it is not eliminated by the kidney's OCT2 transporter. The excretion property of niazirin is shown in Table 4.12.

TABLE 4.12: Excretion property of Niazirin

Property	**Model name**	**Predicted value**
Excretion	Total clearance	1.165
	Renal OCT2 substrate	No

4.5.2.5 Toxicity

From the standpoint of genetic toxicity, niazirin is harmless since it is not mutagenic. The Maximum Human Tolerated Dosage is 0.533 log mg/kg/day this

implies that niazirin is well tolerated by people at appropriate dosages, but higher dosages require caution. Both hERG I and hERG II inhibitors are negative. One extremely good safety aspect of nizazirin is that it is unlikely to create problems with heart rhythm. In animal models, niazirin has minimal acute toxicity of 1.654 mol/kg, indicating a good margin of safety. Chronic Toxicity in Oral Rats LOAEL: 2.139 log mg/kg_bw/day means even when used for an extended period of time in animals, niazirin is generally safe, though extremely high dosages should be avoided. Niazirin is excellent for long-term use because it is not harmful to the liver. It is unlikely that niazirin will result in skin irritation or allergies. Simple aquatic creatures are somewhat poisonous to niazirin, although this amount of toxicity is usually controllable for environmental considerations. Niazirin poses less damage to the environment because it is not highly toxic to fish. The toxicity property of niazirin is shown in Table 4.13.

TABLE 4.13: Toxicity property of Niazirin

Property	**Model name**	**Predicted value**
Toxicity	AMES toxicity	No
	Max. tolerated dose (human)	0.533
	hERG I inhibitor	No
	hERG II inhibitor	No
	Oral Rat Acute Toxicity (LD50)	1.654
	Oral Rat Chronic Toxicity (LOAEL)	2.139
	Hepatotoxicity	No
	Skin Sensitation	No
	T.Pyriformis toxicity	0.301
	Minnow toxicity	3.367

4.5.3 Niazimicin

Like many natural compounds, niazimicin, a naturally occurring chemical identified in *Moringa oleifera*, has potential biological functions but struggles with drug-like qualities. Only a minor amount of niazimicin—probably between 20 and 40 percent—is anticipated to enter the bloodstream when taken orally. The

primary reasons for this restricted bioavailability are that niazimicin may not dissolve well in the stomach, may have trouble effectively passing through intestinal membranes, and may be rapidly degraded by metabolic enzymes such as CYPs and UGTs. Furthermore, it may be actively pumped out of the gut by proteins like P-glycoprotein, which would further restrict absorption.

4.5.3.1 Absorption

With a log mol/L value of -2.76, niazimicin has an intermediate water dissolvability, suggesting that it may dissolve adequately in fluid conditions. Its Caco2 permeability of -0.248 log Papp in 10-6 cm indicates that it has weak permeability across intestinal epithelial cells, which is necessary for oral bioavailability. The gastrointestinal tract moderately transfers the drug into the systemic circulation, as indicated by the moderate intestinal absorption rate of 56.175%. Additionally, its skin permeability, indicated by a log Kp value of -3.111, demonstrates extremely poor dermal absorption, which can help lower systemic exposure when applied topically. The absorption property of niazimicin is shown in table 4.14.

TABLE 4.14: Absorption properties of Niazimicin

Property	**Model name**	**Predicted value**
Absorption	Water solubility	-2.76
	Caco2 permeability	0.248
	Intestinal adsorption (human)	56.175
	Skin permeability	-3.111
	P-glycoprotein substrate	Yes
	P-glycoprotein I inhibitor	No
	P-glycoprotein II inhibitor	No

4.5.3.2 Distribution

Niazimicin is not widely distributed in the body's tissues and does not build up significantly in any one organ. Its free fixation and pharmacological accessibility are impacted by its comparatively good binding to plasma proteins. Its effects on the focused sensory system are limited because of its diminished capacity to cross

the blood-mind barrier. The distribution property of niamizicin is shown in Table 4.15.

TABLE 4.15: Distribution property of Niazimicin

Property	**Model name**	**Predicted value**
Distribution	VDss(human)	-0.404
	Fraction unbound	0.513
	BBB permeability	-0.965
	CNS permeability	-3.504

Niazimicin's VDss of -0.404 (log L/kg) indicates that it is poorly distributed throughout the body. With a fraction unbound (Fu) of 0.513, a significant amount is still in circulation in its active, unbound state. It can cross the blood-brain barrier (log BB-0.965) and has poor permeability to the central nervous system (log PS-3.504). Its distribution characteristics are necessary to comprehend its systemic availability and therapeutic uses.

4.5.3.3 Metabolism

Niazimicin may undergo substantial metabolism in the body, changing into different molecules by oxidation or conjugation, which may have an impact on how long it remains active. The good news is that niazimicin is not expected to be very toxic; at therapeutic dosages, it is probably safe and has little chance of damaging organs or producing genetic damage (non-mutagenic). The metabolism property of niazimicin is shown in Table 4.16.

TABLE 4.16: Metabolism property of Niazimicin

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

It is not anticipated that niazimicin would block CYP1A2, CYP2C19, CYP2C9, or CYP3A4 catalysts, nor will it be a substrate for CYP2D6 or CYP3A4 compounds. These metabolic characteristics suggest that niazimicin is unlikely to significantly influence the metabolism of other medications metabolized by this enzyme due to its favorable pharmacokinetic profile.

4.5.3.4 Excretion

The molecule's calculated total clearance is 0.086 log ml/min/kg, indicating a limited elimination potential. It seems to interact with the renal transport system seldom because it is not an OCT2 substrate. These discharge characteristics may improve its pharmacokinetic profile for therapeutic applications by preserving stable plasma fixations and reducing the possibility of renal aggregation. The excretion property of niazimicin is shown in Table 4.17.

TABLE 4.17: Excretion property of Niazimicin

Property	**Model name**	**Predicted value**
Excretion	Total clearance	0.086
	Renal OCT2 substrate	No

4.5.3.5 Toxicity

The chemical is a viable candidate for more research because of its generally benign toxicological profile. It is safe for the heart (no hERG inhibition), does not result in genetic mutations (AMES negative), and is well tolerated on the skin. Furthermore, it has minimal levels of acute and chronic toxicity in animal models, indicating that it can be administered safely within therapeutic bounds.There is one significant warning indication, though: it appears to be hepatotoxicity positive, which indicates possible liver toxicity. This indicates that although it is generally safe, prolonged usage or greater dosages may have an adverse effect on the liver. Future laboratory and clinical research would need to closely monitor liver function in order to achieve this. With the exception of liver danger, which can be controlled with appropriate dosage and testing, the chemical is generally

safe and effective. It has a lot of promise as a natural remedy, particularly if further research identifies strategies to reduce liver damage. The toxicity property of niazimicin is shown in Table 4.18.

TABLE 4.18: Toxicity property of Niazimicin

Property	**Model name**	**Predicted value**
Toxicity	AMES toxicity	No
	Max. tolerated dose (human)	0.615
	hERG I inhibitor	No
	hERG II inhibitor	No
	Oral Rat Acute Toxicity (LD50)	2.148
	Oral Rat Chronic Toxicity (LOAEL)	2.636
	Hepatotoxicity	Yes
	Skin Sensitation	No
	T.Pyriformis toxicity	0.294
	Minnow toxicity	2.685

4.5.4 Benzyl isothiocyanate

One substance that shows promise for oral use is benzoyl isothiocyanate. It is securely digested by the liver, primarily distributed in the blood, efficiently absorbed from the gut, and doesn't provide significant toxicity hazards to the body. It is a promising therapy candidate for type 1 diabetes, particularly when taken orally, even though it might not be the best option for skin delivery or brain targeting. It works well with combination therapy because it can prevent interactions with liver enzymes.

4.5.4.1 Absorption

The amount that enters the body after oral ingestion may be impacted by benzoyl isothiocyanate's moderate water solubility (log -2.629), which indicates that it may not dissolve readily in water. It can, however, pass through intestinal walls rather easily, according to its Caco-2 permeability value of 1.537, which indicates good intestinal absorption (94.77%). This indicates that when given orally, it has

a high likelihood of being absorbed. Given its limited skin permeability (-1.295), it is unlikely to penetrate readily through the skin. Because it doesn't interact with P-glycoprotein transporters either as an inhibitor or a substrate, benzoyl isothiocyanate is good for preserving its bioavalability. The absorption property of benzyl isothiocyanate is shown in Table 4.19.

TABLE 4.19: Absorption properties of Benzyl isothiocyanate

Property	**Model name**	**Predicted value**
Absorption	Water solubility	-2.629
	Caco2 permeability	1.537
	Intestinal adsorption (human)	94.774
	Skin permeability	-1.295
	P-glycoprotein substrate	No
	P-glycoprotein I inhibitor	No
	P-glycoprotein II inhibitor	No

4.5.4.2 Distribution

The compound's modest volume of distribution (0.193 L/kg) indicates that it mostly remains in the circulation rather than widely dispersing throughout the tissues. According to the fraction unbound (0.379), a sizable amount is still free in the blood and prepared to engage with targets.

Because of its low CNS permeability (logPS -1.862) and poor brain permeability (logBB -0.45), it is unlikely to enter the brain or have an impact on the central nervous system. The distribution property of benzyl isothiocyanate is shown in Table 4.20.

TABLE 4.20: Distribution property of Benzyl isothiocyanate

Property	**Model name**	**Predicted value**
Distribution	VDss(human)	-0.193
	Fraction unbound	0.379
	BBB permeability	0.45
	CNS permeability	-1.862

4.5.4.3 Metabolism

CYP3A4, CYP2D6, and CYP2C9 are examples of important metabolic enzymes that do not seem to be substrates or inhibitors of benzoyl isothiocyanate. This indicates a decreased chance of metabolic medication interactions, which is encouraging. Table 4.21 shows metabolism property of benzyl isothiocyanate.

TABLE 4.21: Metabolism property of Benzyl isothiocyanate

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

4.5.4.4 Excretion

Its moderate overall clearance (0.305 mL/min/kg) suggests a respectable rate of bodily removal. It is not a substrate for the renal OCT2 transporter, indicating that the kidneys won't need this pathway to eliminate it. Excretion property of benzyl isothiocyanate is shown in Table 4.22.

TABLE 4.22: Excretion property of Benzyl isothiocyanate

Property	**Model name**	**Predicted value**
Excretion	Total clearance	0.305
	Renal OCT2 substrate	No

4.5.4.5 Toxicity

Since benzoyl isothiocyanate does not exhibits AMES toxicity, it is unlikely to result in cancer or genetic abnormalities. The approved maximum tolerated dose is 0.814 log mg/kg/day. Heart toxicity (hERG inhibition) is not a risk factor.

At larger dosages, however, there may be some toxicity, according to its oral rat acute toxicity LD50 (2.254) and chronic toxicity LOAEL (1.777) values. It has comparatively low T. Pyriformis and Minnow toxicity scores, indicating low environmental toxicity, and it does not produce skin sensitivity. The toxicity of benzyl isothiocyanate is shown in Table 4.23.

TABLE 4.23: Toxicity property of Benzyl isothiocyanate

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)	2.254
	Oral Rat Chronic Toxicity (LOAEL)	1.777
	Hepatotoxicity	No
	Skin Sensitation	Yes
	T.Pyriformis toxicity	1.059
	Minnow toxicity	1.281

4.6 Sitosterol

A naturally occurring plant component with a strong ADMET profile is sitosterol. Although it has a high intestinal absorption rate, its low water solubility and P-glycoprotein recognition may limit the amount that eventually enters the bloodstream. Although sitosterol's real effects on the central nervous system are likely minimal, it primarily remains in the circulation once it enters the body, with limited distribution to tissues and some capacity to pass the brain barrier. It is unlikely to result in significant drug interactions because it doesn't significantly alter important liver enzymes.

Its great safety profile includes being non-toxic, non-mutagenic, and non-hepatotoxic, with just a minor risk for possible cardiac rhythm effects (hERG II). It is also eliminated at a slow pace, primarily by the liver. Sitosterol seems to be a safe and

promising natural substance overall, although its formulation might need to be improved to get past problems with P-gp and solubility.

4.6.0.1 Absorption

Due to its extremely low water solubility (-6.063 log mol/L), sitosterol may not be absorbed to the fullest extent possible in the body's watery environment. However, its Caco-2 permeability is moderate (1.2 log Papp), and intestinal absorption is rather good (about 94.938% absorbed), demonstrating that even if it's poorly soluble, the chemical can still get into the body fairly efficiently, possibly through specialized transport pathways. It is not very effective for skin applications because of its limited skin permeability (-2.737 log Kp). The absorption property of sitosterol is shown in Table 4.24.

TABLE 4.24: Absorption properties of Sitosterol

Property	**Model name**	**Predicted value**
Absorption	Water solubility	-6.063
	Caco2 permeability	1.2
	Intestinal adsorption (human)	94.983
	Skin permeability	-2.737
	P-glycoprotein substrate	No
	P-glycoprotein I inhibitor	Yes
	P-glycoprotein II inhibitor	Yes

4.6.0.2 Distribution

About 41% of sitosterol is unbound in the blood, meaning a significant amount is free to interact with the body's tissues. Sitosterol also tends to remain primarily in the bloodstream (VDss -0.108 log L/kg). It's interesting to note that sitosterol has a BBB permeability of 0.813, which indicates some possibility for crossing the blood-brain barrier. However, its CNS permeability of -1.435 log PS indicates that it may still have limited penetration into brain regions. This suggests that although it may have some degree of brain penetration, its effects on the central

nervous system may not be profound. The distribution property of sitosterol is shown in Table 4.25.

TABLE 4.25: Distribution property of Sitosterol

Property	**Model name**	**Predicted value**
Distribution	VDss(human)	-0.108
	Fraction unbound	0
	BBB permeability	0.813
	CNS permeability	-1.435

4.6.0.3 Metabolism

Sitosterol interacts with CYP3A4 enzymes for metabolism (it is a substrate), which means that this vital liver enzyme can digest it. However, it does not inhibit key CYP enzymes such as CYP1A2, CYP2D6, or CYP2C9. This is usually a positive indication because it shows that sitosterol won't affect how other medications are metabolized, which makes it safer to take with other medications. The metabolism property of sitosterol is shown in Table 4.26.

TABLE 4.26: Metabolism property of Sitosterol

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

4.6.0.4 Excretion

With a moderate overall clearance (0.621 mL/min/kg), sitosterol leaves the body at a balanced rate rather than too soon or too slowly. Since the renal OCT2 transporter does not excrete it, additional routes, such as bile, are probably involved. The excretion property of sitosterol is shown in Table 4.27.

TABLE 4.27: Excretion property of Sitosterol

Property	**Model name**	**Predicted value**
Excretion	Total clearance	0.621
	Renal OCT2 substrate	No

4.6.0.5 Toxicity

Because it is not mutagenic (no AMES toxicity) and has a tolerable maximum tolerated dose (-0.67 log mg/kg/day), sitosterol has a clean profile. Since it doesn't block hERG channels, it is unlikely to interfere with heart rhythm, which is a crucial safety precaution. There is no risk of hepatotoxicity or skin sensitization, and it exhibits low acute (LD50: 2.783 mol/kg) and chronic toxicity (LOAEL: 0.847 log mg/kg/day). It does, however, exhibit mild minnow toxicity (-1.919 mM) and moderate toxicity to T. pyriformis (0.323 µg/L), indicating some ecological impact but nothing concerning at normal concentrations. The toxicity property of sitosterol is shown in Table 4.28.

TABLE 4.28: Toxicity property of Sitosterol

Property	**Model name**	**Predicted value**
Toxicity	AMES toxicity	No
	Max. tolerated dose (human)	-0.67
	hERG I inhibitor	No
	hERG II inhibitor	Yes
	Oral Rat Acute Toxicity (LD50)	2.783
	Oral Rat Chronic Toxicity (LOAEL)	0.847
	Hepatotoxicity	No
	Skin Sensitation	No
	T.Pyriformis toxicity	0.323
	Minnow toxicity	-1.919

4.6.1 Gallic Acid

Although gallic acid is harmless and highly soluble in water, its absorption is limited; it dissolves readily but has trouble entering the bloodstream. After entering,

it remains in the bloodstream and does not stray into the brain or adipose tissue. Because of its low toxicity concerns and lack of interference with the body's natural detox systems (CYP enzymes), it is a safe, helpful antioxidant for therapeutic usage.

4.6.1.1 Absorption

The compound's anticipated value of -2.56 indicates that its water solubility is moderate.Its low absorption potential is indicated by its poor permeability across Caco-2 cells, as seen by its log Papp value of -0.081. Under intestinal conditions that are mimicked, its absorption rate is moderate, at 43.374%. Furthermore, the compound's log Kp value of -2.735 shows that it cannot pass through the epidermal barrier. It has a limited likelihood of forming connections with P-glycoprotein (P-gp), P-glycoprotein I, or P-glycoprotein II because it is not a substrate for any of these vehicle proteins. The absorption property of gallic acid is shown in Table 4.29.

TABLE 4.29: Absorption properties of Gallic acid

Property	**Model name**	**Predicted value**
Absorption	Water solubility	-2.56
	Caco2 permeability	-0.081
	Intestinal adsorption (human)	43.374
	Skin permeability	-2.735
	P-glycoprotein substrate	No
	P-glycoprotein I inhibitor	No
	P-glycoprotein II inhibitor	No

4.6.1.2 Distribution

Following absorption, gallic acid is distributed in a reasonable volume across the body's tissues, suggesting that it does not build up significantly in any one organ. gallic acid influences the free focus and accessibility of plasma proteins for pharmacological actions by binding to them in a reasonable manner. Its restricted capacity to cross the blood-cerebrum barrier limits the effects it can have on the

focused sensory system. The distribution property of gallic acid is shown in Table 4.30.

TABLE 4.30: Distribution property of Gallic acid

Property	**Model name**	**Predicted value**
Distribution	VDss(human)	-1.855
	Fraction unbound	0.617
	BBB permeability	-1.102
	CNS permeability	-3.74

A VDss of -1.855 (log L/kg) indicates that gallic acid has very little dispersion throughout the body.With a fraction unbound (Fu) of 0.617, a sizable amount is still in circulation in its active, unbound condition. It cannot cross the blood-brain barrier (log BB-1.102) and has poor permeability to the central nervous system (log PS-3.74). Its distribution characteristics are necessary to comprehend its systemic availability and therapeutic uses.

4.6.1.3 Metabolism

Because it doesn't inhibit any of the major liver enzymes (CYP3A4, CYP2D6, CYP2C9, etc.), gallic acid has a highly favorable metabolic profile. Gallic acid is unlikely to disrupt the metabolism of other medications, which is fantastic. It behaves in the liver's metabolic system like a well-behaved guest. The metabolism property of gallic acid is shown in Table 4.31.

TABLE 4.31: Metabolism property of Gallic acid

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

4.6.1.4 Excretion

Although it remains within a reasonable range, gallic acid's poor clearance rate (around 0.518 mL/min/kg) indicates that it remains in the body for a little while longer before being removed. This implies that it may be effectively processed and eliminated by the body without remaining too long and building up. The excretion property of gallc acid is shown in Table 4.32.

TABLE 4.32: Excretion Property of Gallic acid

Property	**Model name**	**Predicted value**
Excretion	Total clearance	0.518
	Renal OCT2 substrate	No

4.6.1.5 Toxicity

Since Gallic acid is expected to be non-mutagenic, there is no chance that it may cause cancer. With a moderate LD50 value and a moderate maximum tolerated dose, it shows some toxicity at high dosages. Crucially, it doesn't block hERG channels, therefore heart problems are unlikely to result. It doesn't induce skin sensitivity or hepatotoxicity. It does, however, exhibit some toxicity in aquatic models (minnows and T. pyriformis), hence environmental exposure should be done with caution. The toxicity propert of gallic acid is shown in Table 4.33.

TABLE 4.33: Toxicity property of Gallic acid

Property	**Model name**	**Predicted value**
Toxicity	AMES toxicity	No
	Max. tolerated dose (human)	0.7
	hERG I inhibitor	No
	hERG II inhibitor	No
	Oral Rat Acute Toxicity (LD50)	2.218
	Oral Rat Chronic Toxicity (LOAEL)	3.06
	Hepatotoxicity	No
	Skin Sensitation	No
	T.Pyriformis toxicity	0.285
	Minnow toxicity	3.188

4.7 Lipinski Rule of Five

Early on in the drug design process, medicinal chemists have found great success using Lipinski's Rule of Five. According to the rule, compounds are more likely to have favorable pharmacokinetic properties if they have no more than one violation of the following criteria:

1. No more than five hydrogen bond donors.
2. No more than ten hydrogen bond acceptors.
3. A molecular weight less than 500 Da, and
4. A calculated log P (lipophilicity) of less than 5.
5. The number of rotatory bonds should be less than five.

As a result, our compound is run in accordance with the rules, enabling the investigation of different *Moringa oleifera* binding agents. The outcomes are displayed in Table 4.34.

TABLE 4.34: Physiochemical properties of Moringa oleifera ligands

Ligands	**Log P value**	**Molecular weight**	**Hydrogen bond donors**	**Hydrogen bond acceptors**	**Rotatory bonds**
Moringin	0.4957	311.359	3	7	4
Niazirin	-0.04102	279.292	3	6	3
Niazimicin	0.3039	357.428	4	7	5
Benzyl isothiocyanate	2.2894	149.218	0	2	2
Sitosterol	8.1047	416.734	1	1	6
Gallic acid	0.5016	170.12	4	4	1

The molecular weight, lipophilicity (logP), and capacity to participate in hydrogen bonding interactions (H-bond donors and acceptors) are among the attributes that shed light on each ligand's chemical makeup. These elements are essential

to comprehending their possible pharmacological characteristics and biological actions.

Along with the molecular weight and logP values, the table displays the donor and acceptor of the hydrogen bond. *Moringa oleifera* ligand values. Orally active chemicals will adhere to these requirements. Depending on how it is taken, it can have a drug-like effect. If a material satisfies three or more standards, it is considered a medication; if it doesn't, it is considered little known. Nearly all ligands adhered to the Lipinski criterion of five.

4.8 Molecular Docking

The protein and ligand files must be properly prepared in order to run molecular docking simulations. SDF format, which enables the depiction of molecular structures with atom-by-atom detail, was used to save the ligand structures. However, the protein structure was recorded in PDB format, which is a common file format for displaying three-dimensional protein and other macromolecule structures.

These modified protein and ligand files were submitted to CB Dock 2 with flawless filenames and no gaps. The molecular docking program CB Dock 2 forecasts a ligand's preferred orientation upon binding to a protein receptor. It assists in forecasting the most energetically advantageous binding mode between the ligand and the protein by examining several conformations and orientations.

The receptor for ligand binding is provided by the protein structure, which has been refined and optimized with PyMOL, and the ligand structures are possible therapeutic candidates. Ligands were refined and optimized by minimizing their energies using Chem 3D pro. In order to anticipate the ligands' binding affinities and orientations, CBDock 2 use algorithms to investigate the binding interactions between the ligands and the protein's active sites.

The ligand-protein complexes' conformations are arranged according to their binding affinities in molecular docking. The greatest affinity score, which represents the strength of the protein-ligand interaction, is used to select the ideal shape.

The docked structures that are produced after the docking procedure are chosen for additional examination. By analyzing docking scores, cavity sizes, grid maps, and binding energies, the best-docked structure is found. These standards aid in identifying the ligand-protein interaction that has the greatest promise for further research.

The lead and standard compounds were docked against the target proteins, and the best binding score was obtained from the docking result.

4.8.1 Docking Complex with Moringin

Moringin exhibits a weak binding affinity with the target protein in the CB-Dock 2 molecular docking analysis, with a Vina score of -5.3. The ligand does not fits well into the docking site, as indicated by the binding cavity's volume of 544 A3, and the docking box's dimensions (20× 20 × 20 A3) are suitable for efficient docking. The moringin docking complex is displayed in Figure 4.5.

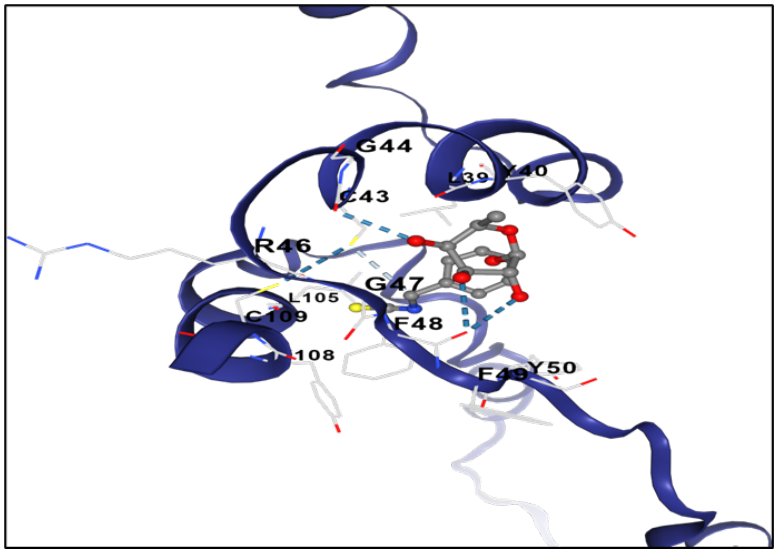


FIGURE 4.5: Dock complex of Moringin with *INS*

4.8.2 Docking Complex with Niazirin

With a Vina score of -6.0, niazirin has a moderate binding affinity. This niazirin binds to the target protein efficiently. There may be some flexibility in the binding

interactions because the binding cavity volume, 55A3. With its dimensions of 20 × 20× 20 A3, the docking box offers sufficient room for docking.The docking complex of niazirin is displayed in Figure 4.6.

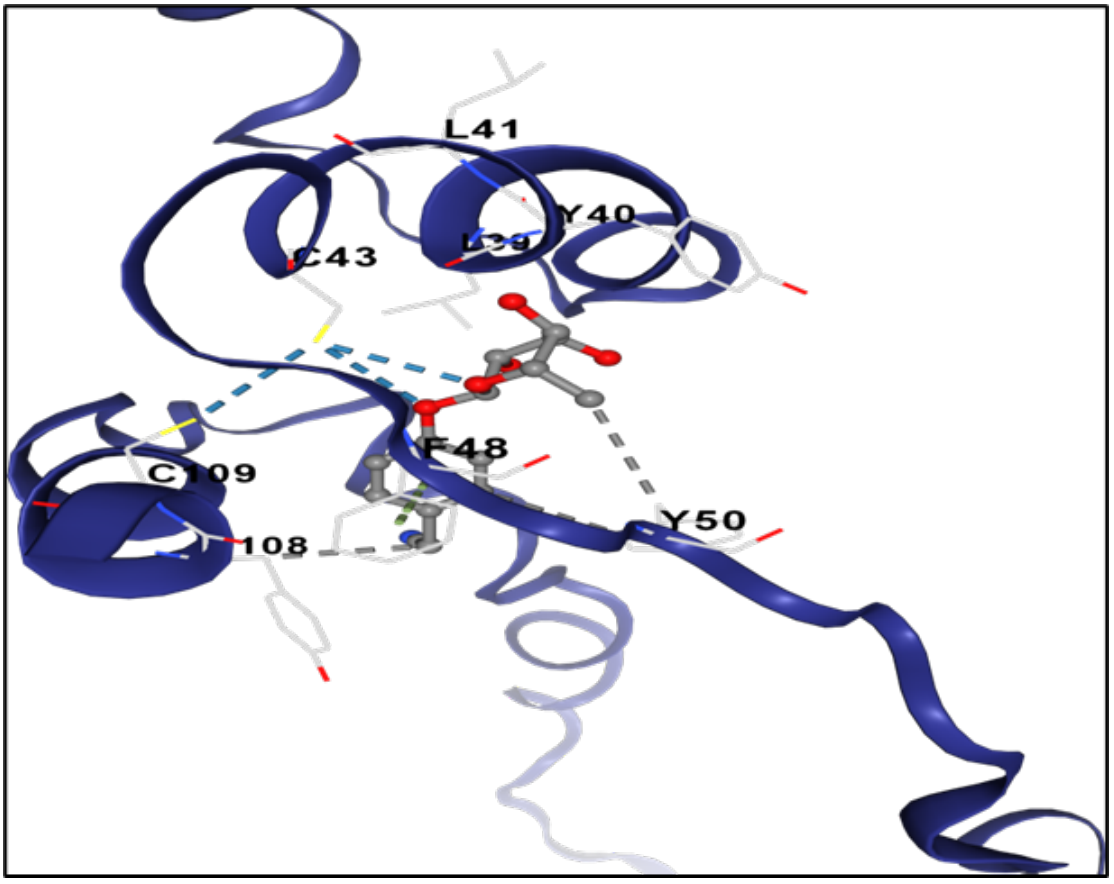


FIGURE 4.6: Dock complex of niazirin with *INS*s

4.8.3 Docking Complex with Niazimicin

With a Vina score of -5.9, niazimicin is anticipated to have a moderate binding affinity. According to this score, niazimicin might attach to the target protein more successfully. A tighter and maybe more precise interaction with the ligand may result from the comparatively smaller binding cavity volume of 544 A3. The dimensions of the docking box (20 × 20 × 20 A3) are suitable for the docking procedure. The niazimicin docking complex is displayed in Figure 4.7.

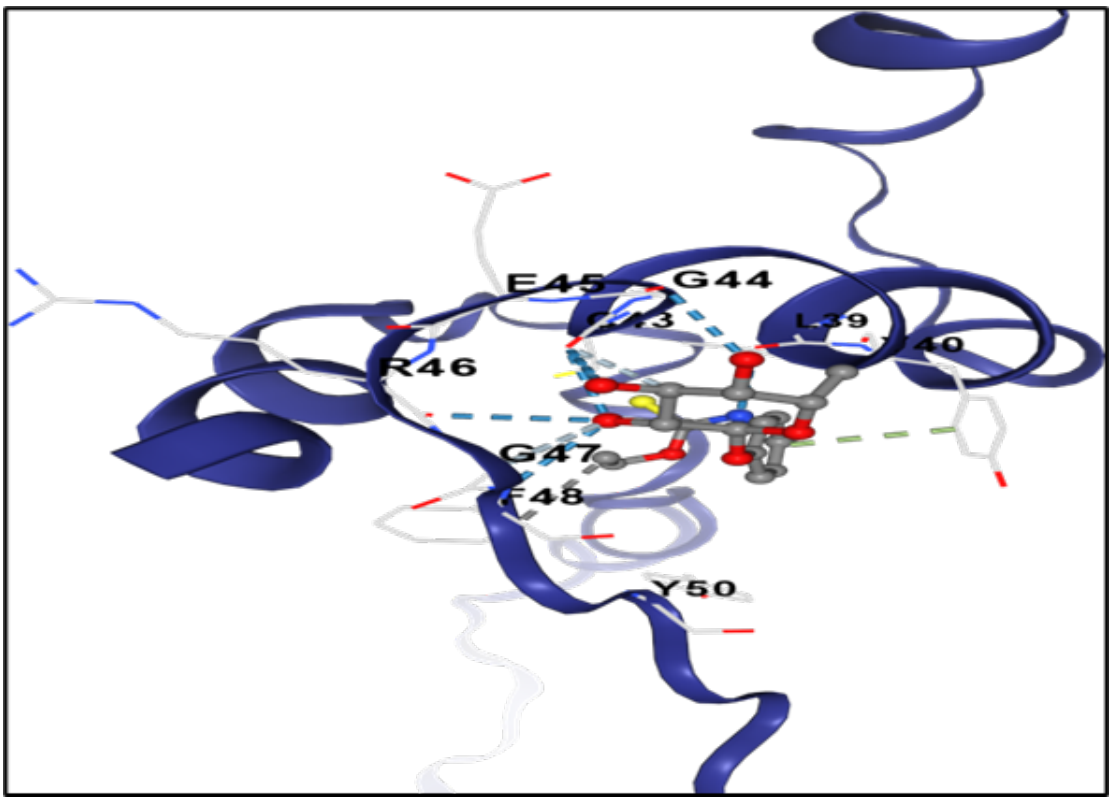


FIGURE 4.7: Dock complex of niazimicin with *INS*

4.8.4 Docking Complex with Benzyl isothiocyanate

With the most highest Vina score of -8.0 among the ligands in the list, benzyl isothiocyanate is expected to have the highest binding affinity to the target protein. This implies that, in comparison to the other chemicals, benzyl isothiocyanate is probably going to bond the best.

This implies that, in comparison to the other chemicals, benzyl isothiocyanate is probably going to bond the best.

The binding cavity volume is 544 A3, which permits a potentially flexible binding relationship. With its dimensions of 26 × 26 × 26 A3, the docking box offers plenty of room for the docking procedure.

The benzyl isothiocyanate docking complex is displayed in Figure 4.8.

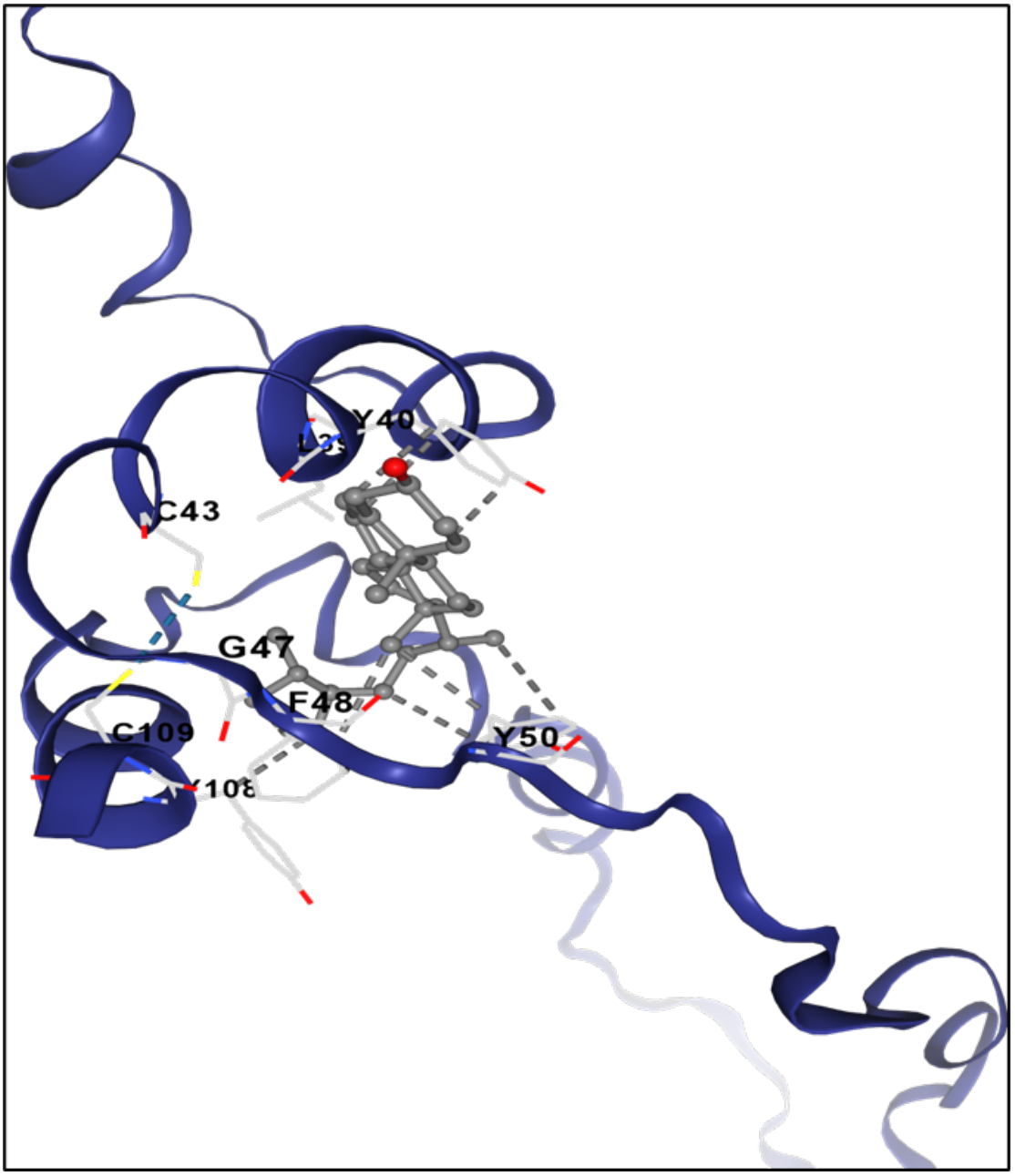


FIGURE 4.8: Dock complex of Benzyl isothiocyanate with *INS*

4.8.5 Docking complex with Sitosterol

Sitosterols exhibits a very strong binding affinity with the target protein in the CB-Dock 2 molecular docking analysis, with a Vina score of -7.5. The ligand fits well into the docking site, as indicated by the binding cavity's volume of 544 A3, and the docking box's dimensions (25 × 25 × 25 A3) are suitable for efficient docking. The sitosterol docking complex is displayed in Figure 4.9.

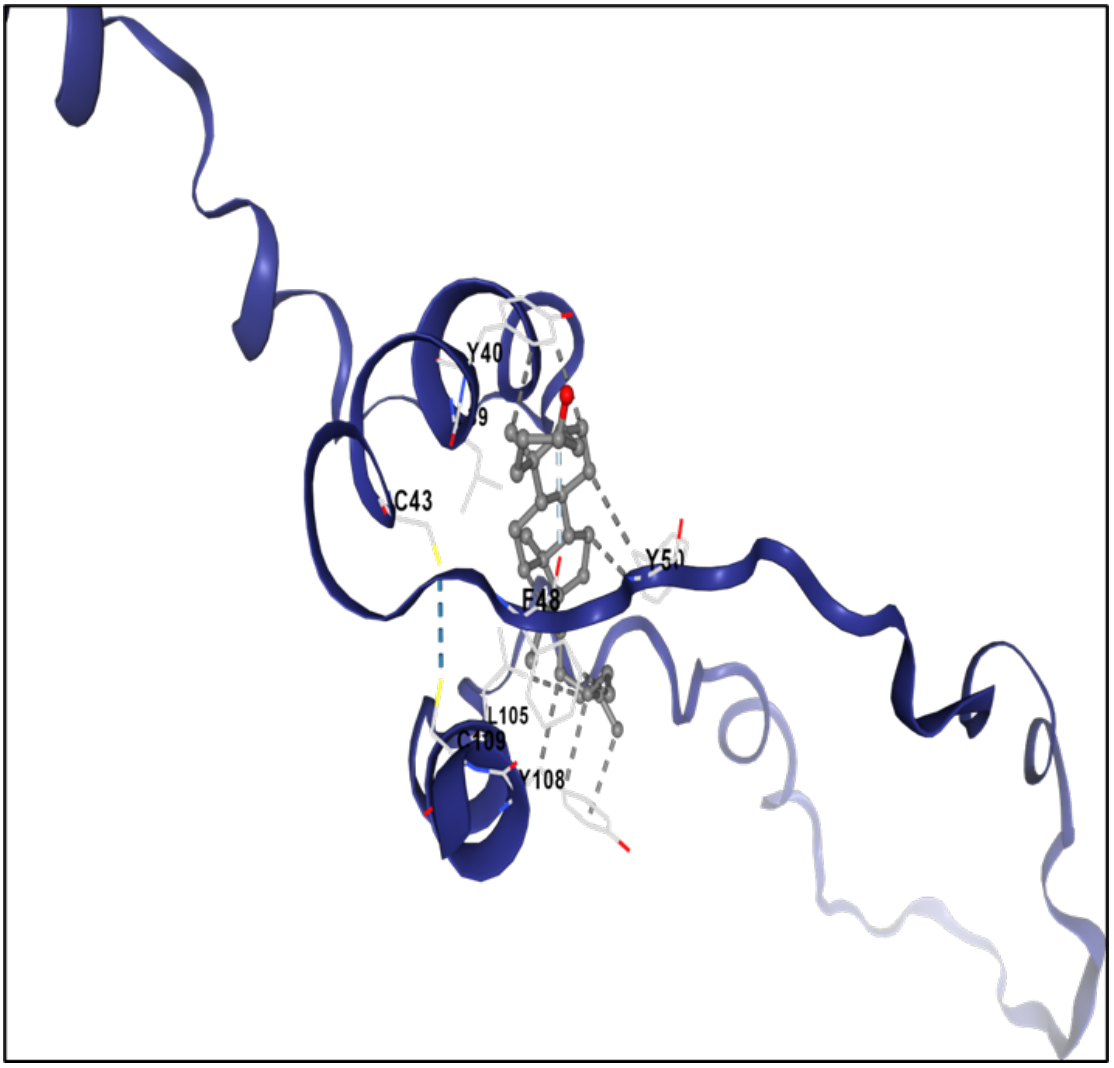


FIGURE 4.9: Dock complex of Sitosterol with *INS*

4.8.6 Docking Complex with Gallic Acid

Gallic acid has the weakest binding affinity with the target protein in the CB-dock 2 molecular docking analysis, with a Vina score of -4.7.

The ligand does not fits well into the docking site, as indicated by the binding cavity's volume of 226A3, and the docking box's dimensions (17× 17 × 17 A3) are suitable for efficient docking.

The gallic acid docking complex is displayed in Figure 4.10.

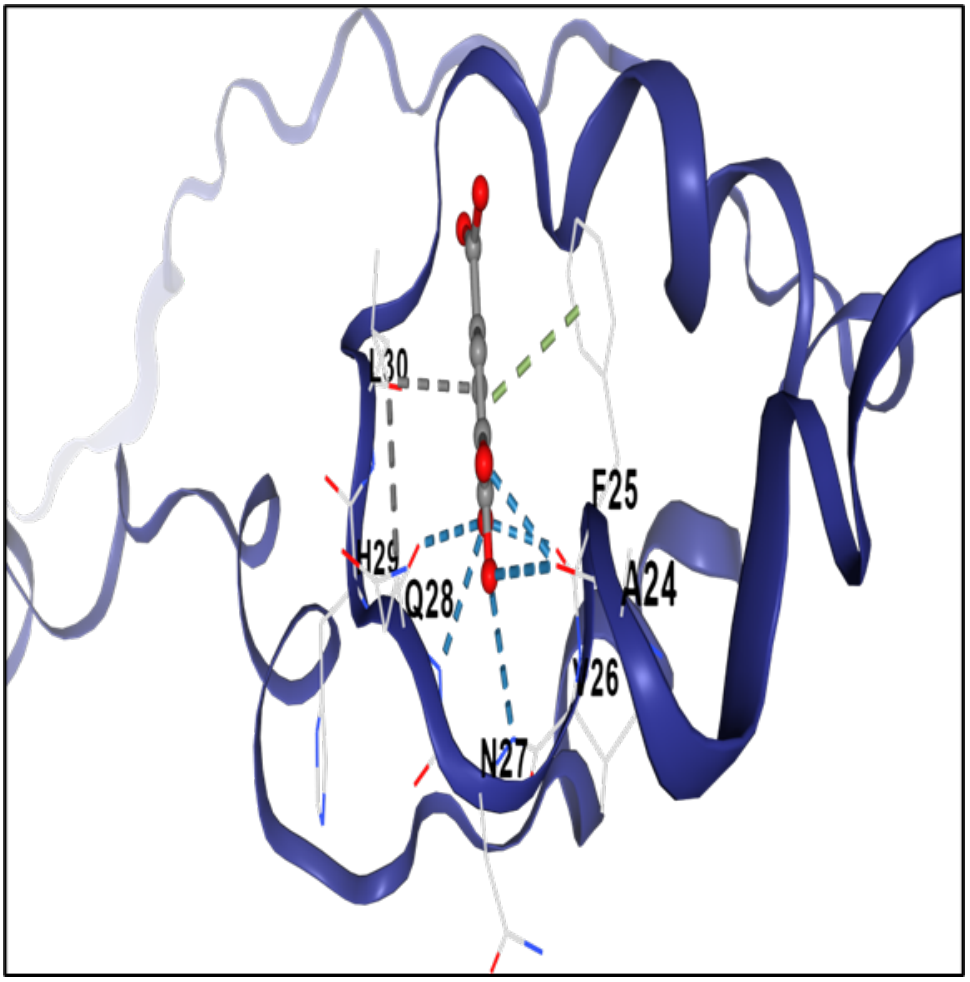


FIGURE 4.10: Dock complex of Gallic Acid with *INS*

The docking characteristics of different ligands, such as Vina scores, cavity volumes, center coordinates, and docking sizes, are shown in table 4.35.

TABLE 4.35: Docking Properties of ligands

Ligand	**Vina Score**	**Cavity Volume (A^3)**	**Center (x,y,z)**	**Docking Size (x,y,z)**
Moringin	-5.3	544	4,-7,-1	20,20,20
Niazirin	-6	55	3,-3,2	20,20,20
Niazimicin	-5.9	544	4,-7,-1	20,20,20
Benzyl isothiocyanate	-8	544	4,-7,-1	26,26,26
Sitosterol	-7.5	544	4,-7,-1	25,25,25
Gallic Acid	-4.7	226	-12,6,-3	17,17,17

4.9 Ligplot Analysis

A appreciated computer program for drawing schematic diagrams of protein-ligand interactions is called Ligplot. It makes it simpler to analyze and understand the binding mechanisms by providing a clear and accurate image of the interactions between a protein and a ligand.

It makes it simpler to analyze and understand the binding mechanisms by providing a clear and accurate image of the interactions between a protein and a ligand. The protein-ligand complex's stability and selectivity depend on hydrogen bonding and hydrophobic interactions, which LIGPLOT illustrates. Using specific ligands from PDB files, this software creates schematic representations of protein-ligand interactions.

Using specific ligands from PDB files, this software creates schematic representations of protein-ligand interactions. In order to examine hydrogen and hydrophobic bonding, the docked data were submitted in PDB format. There were several hydrogen bonding and hydrophobic interactions between the target protein and the six ligands. The interactions between the ligands and the amino acid residues of proteins will be shown using LigPlot, which will produce schematic 3D diagrams that emphasize polar bonds, hydrogen bonds, and hydrophobic interactions.

Since these interactions have a major impact on the ligand's binding affinity and specificity, identifying polar bonds is very crucial. We can assess the strength and importance of these polar contacts in stabilizing the ligand-protein by examining them. This thorough investigation will enhance our comprehension of the molecular underpinnings of ligand-receptor interactions and provide insights into the crucial residues involved in ligand binding.

Significant hydrophobic and hydrogen bonding interactions occur in ligand-receptor complexes. The ligand-receptor interactions are shown in the following diagrams.

The interaction between moringin and receptor protein is depicted in Figure 4.11.

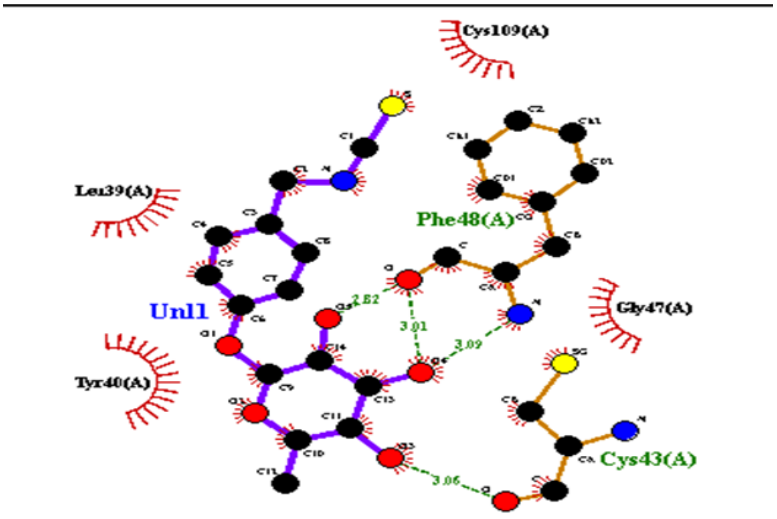


FIGURE 4.11: Interaction of Moringin

The figure 4.11 demonstrates that four hydrogen bonds and four hydrophobic interactions have been established by moringin.

Figure 4.12 shows the interaction between niazirin and receptor protein.

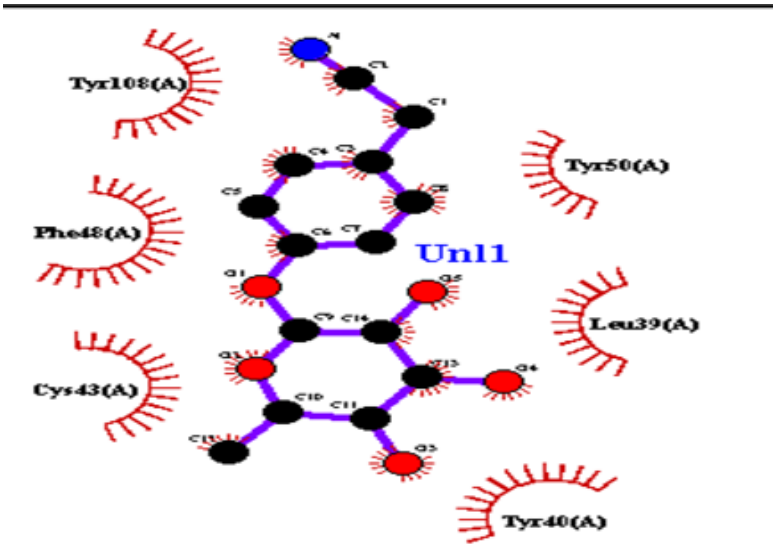


FIGURE 4.12: Interaction of Niazirin

The figure 4.12 shows that niazirin has formed six hydrophobic contacts and zero hydrogen bonds.

The interaction between niazimicin and the receptor protein is depicted in Figure 4.13.

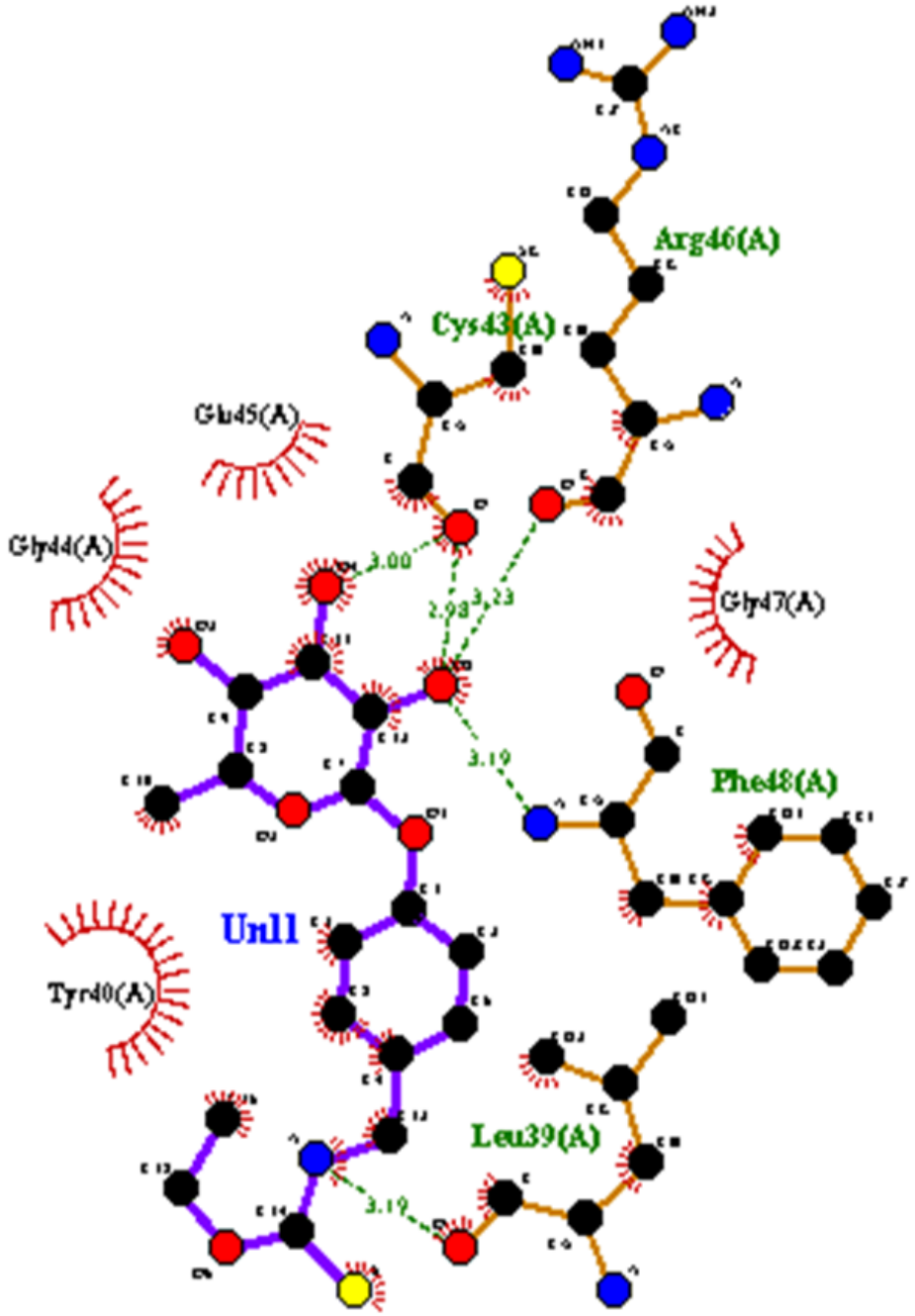


FIGURE 4.13: Interaction of Niazimicin

The interaction between niazimicin and the receptor protein is depicted in Figure 4.13. It demonstrates that niazimicin contains five hydrogen bonds and four hydrophobic interactions.

The interaction between benzyl isothiocyanate and the receptor protein is depicted in Figure 4.14.

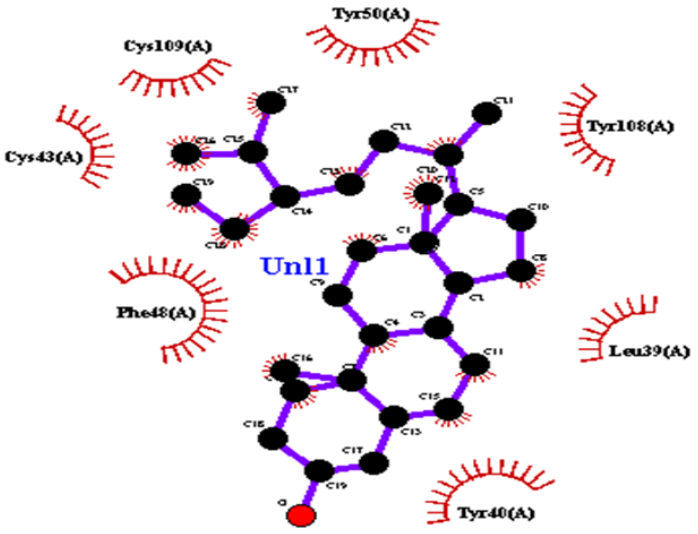


FIGURE 4.14: Interaction of Benzyl isothiocyanate

Figure 4.14 above illustrates how benzyl isothiocyanate and protein interact. It has zero hydrogen bonds and seven hydrophobic contacts.

The interaction between benzyl isothiocyanate and the receptor protein is depicted in Figure 4.15.

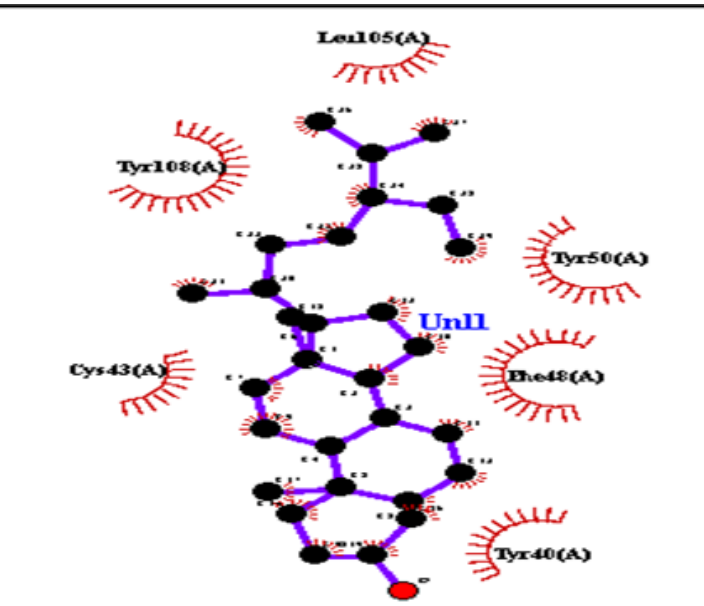


FIGURE 4.15: Sitosterol

Figure 4.15 shows the interaction of Sitosterol with target protein.it has six hydrophobic interactions only.

The interaction between gallic acid and protein is depicted in Figure 4.16.

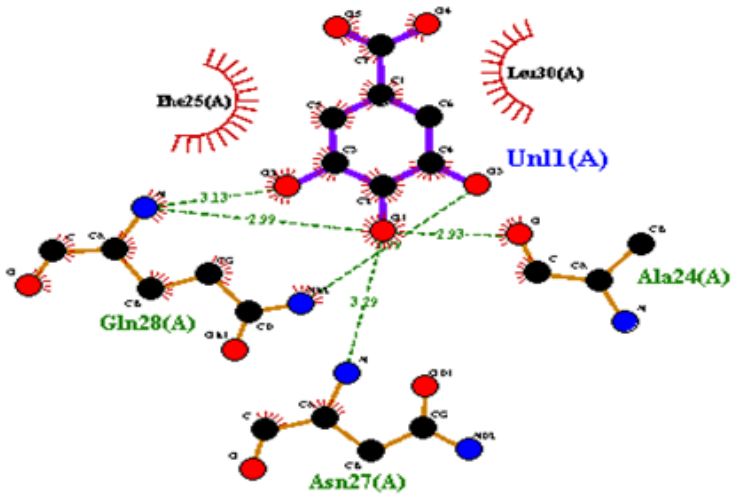


FIGURE 4.16: Gallic acid

The interaction between gallic acid and protein is depicted in Figure 4.16. It has five hydrogen bonds, two hydrophobic contacts, and three amino acids.

Table 4.36 displays the properties of compounds derived by ligplots, such as amino acid, hydrogen bonding, and hydrophobic bonding.

TABLE 4.36: Properties of Compound Interactions as Displayed by LigPlot

Ligands	**Amino Acid**	**Hydrogen Bond Distance**	**Hydrophobic Interactions**
Moringin	1.Cys43 (A)	2.62	Cys109 (A)
	2.Phe48 (A)	3.01	Leu39 (A)
		3.09	Gly47 (A)
		3.06	Tyr40 (A)
Niazirin	None	None	Tyr108 (A)
			Tyr50 (A)
			Phe48 (A)
			Leu39(A)
			Cys43 (A)

continued on next page

Table 4.36 continued from previous page

Ligands	**Amino Acid**	**Hydrogen Bond Distance**	**Hydrophobic Interactions**
			Tyr40 (A)
Niazimicin	1.Arg46 (A)	3	Glu45 (A)
	2.Cys43(A)	2.98	Glydd(A)
	3.Phe48 (A)	3.23	Tyr40 (A)
	4.Leu39 (A)	3.19	Gly47 (A)
		3.19	
Benzyl isothiocyanate	None	None	Tyr50 (A)
			Cys109 (A)
			Cys43 (A)
			Phe48 (A)
			Tyr40 (A)
			Leu39 (A0
			Tyr108 (A)
Sitosterol	None	None	Leu105 (A)
			Tyr108 (A)
			Cys43 (A)
			Tyr50 (A)
			Ehe48 (A)
			Tyr40 (A)
Gallic Acid	1.Gln28 (A)	3.13	Leu30 (A)
	2.Ala24 (A)	2.99	Ehe25 (A)
	3.Asn27 (A)	2.93	
		3.29	
		2.99	

4.10 Lead Compound Identification

Compounds' ultimate fate as drugs or non-drugs is dictated by their physiochemical and pharmacokinetic characteristics.Pharmacokinetic studies serve as a secondary filter in the screening of the possible compounds, while physicochemical qualities, or Lipinski rule, function as a main filter. Based on their physicochemical characteristics, docking score, and Lipinski rule of five, benzyl isothiocyanate,

sitosterol, and niazirin are taken into consideration for additional screening. Others, however, are eliminated at the initial screening.and benzyl isothiocyanate is chosen as the lead compounddue to its good absorption, moderate distribution, safe metabolism, controlled clearance and low toxicity.

4.11 Reference Drug Identification

The best anti-diabetic medication is chosen based on its physicochemical and ADME/T characteristics, as well as its mode of action and adverse effects. The pkCSM online tool is used to evaluate the ADME/T characteristics, while the PubChem online database is used to retrieve the physicochemical properties. The DrugBank and KEGG are used to determine the mechanism of action. The DrugBank and KEGG are used to determine the mechanism of action.

4.11.1 Metformin Mechanism of Action

Metformin reduces the amount of glucose the liver produces, which raises insulin sensitivity. By triggering AMP-activated protein kinase (AMPK), which inhibits gluconeogenic enzymes, it lowers the amount of glucose produced by the liver. Additionally, metformin enhances glucose absorption in muscle and adipose tissues by promoting GLUT4 translocation to the cell membrane.

Blood sugar levels may also drop as a result of altered gut flora and decreased intestinal glucose absorption impact.

Although metformin can occasionally result in lactic acidosis and gastrointestinal side effects, it is an effective medication for reducing blood glucose levels without causing severe hypoglycemia.

Because of its efficient mode of action and good safety record, metformin is a perfect reference medication for comparative research, offering a strong basis for assessing novel therapeutic agents like protocatechuic acid in relation to glucose metabolism and IRS protein sensitivity [78].

4.12 Drug ADMET Properties

4.12.1 Absorption Property Comparision

Both substances are somewhat soluble in water, which is comparable to their water solubility. Although metformin is slightly less soluble than benzoyl isothiocyanate, the negative logS suggests restricted solubility.Compared to metformin, benzoyl isothiocyanate exhibits significantly higher Caco-2 permeability, which indicates that it can pass through intestinal cell membranes more readily and may have a higher potential for oral absorption.The intestinal absorption of benzyl isothiocyanate is over 95%, but that of metformin is only roughly 59%. This implies that benzyl isothiocyanate would probably reach the bloodstream more effectively than metformin upon oral ingestion. Because it is not impacted by P-glycoprotein efflux, has a higher intestinal absorption, and crosses membranes more readily, Benzyl isothiocyanate appears to be a superior absorber.Since metformin is a P-gp substrate, it may be pumped out of cells, which in some situations may reduce its efficiency. There is not much of a difference between the two compounds' moderate water solubility. The absorption property of both reference drug and the lead compound is shown in Table 4.37.

TABLE 4.37: Absorption properties of drug and lead compound

Property	**Model name**	**Predicted Value of Metformin**	**Predicted Value of Benzyl isothiocyanate**
Absorption	Water solubility	-2.707	-2.629
	Caco2 permeability	-0.339	1.537
	Intestinal adsorption (human)	59.401	94.774
	Skin permeability	-2.735	-1.295
	P-glycoprotein substrate	Yes	No
	P-glycoprotein I inhibitor	No	No
	P-glycoprotein II inhibitor	No	No

4.12.2 Distribution Property Comparision

Benzyl Isothiocyanate has a moderate tissue distribution, can penetrate the blood-brain barrier, and can enter the brain. Less is "free" in the blood, though, as it is primarily bound to plasma proteins (lower fraction unbound). With a high free fraction available for action and low brain penetration, metformin primarily remains in the blood plasma. The distribution property of both reference drug and the lead compound is shown in Table 4.38.

TABLE 4.38: Distribution properties of drug and lead compound

Property	**Model Name**	**Predicted Value of Metformin**	**Predicted Value of Benzyl isothiocyanate**
Distribution	VDss(human)	-0.232	-0.193
	Fraction unbound	0.811	0.379
	BBB permeability	-0.946	0.45
	CNS permeability	-4.238	-1.862

4.12.3 Metabolism Property Comparision

According to the compound's metabolic properties, it is not a substrate for the CYP2D6 or CYP3A4 enzymes. Moreover, it has no effect on the CYP1A2 or CYP3A4 enzymes. This implies that the molecule's ability to interact metabolically with these compounds may be limited. The metabolism property of both reference drug and the lead compound is shown in Table 4.39.

TABLE 4.39: Metabolism Property of drug and lead compound

Property	**Model Name**	**Predicted Value of Metformin**	**Predicted Value of Benzyl isothiocyanate**
Metabolism	CYP2D6 substrate	No	No
	CYP3A4 substrate	No	No
	CYP1A2 inhibitor	No	No
	CYP2C19 inhibitor	No	No
	CYP2C9 inhibitor	No	No
	CYP2D6 inhibitor	No	No
	CYP3A4	No	No

4.12.4 Excretion Property Comparision

Benzyl isothiocyanate has a shorter half-life than metformin because it is eliminated from the body more quickly. Its excretion is independent of OCT2, which is advantageous if you are concerned about drug-drug interactions.However, metformin does not need OCT2 for elimination and remains in the body for a longer period of time, extending its window of action. The excretion property of both reference drug and the lead compound is shown in Table 4.40.

TABLE 4.40: Excretion Property of drug and lead compound

Property	**Model Name**	**Predicted Value of Metformin**	**Predicted Value of Benzyl isothiocyanate**
Excretion	Total clearance	0.1	0.305
	Renal OCT2 substrate	No	No

4.12.5 Toxicity Property Comparision

Benzyl isothiocyanate has good cardiac safety, is safe to use at modest dosages, and is not mutagenic. However, compared to metformin, it has a little higher acute and longterm toxicity and may irritate the skin. Metformin has minimal toxicity to the liver and heart, however it has mutagenic potential in AMES tests. Additionally, it is more harmful to aquatic life, such as minnows, in the environment. At typical dosages, both substances are rather safe, however they both have the potential of causing skin irritation. The toxicity property of both reference drug and the lead compound is shown in Table 4.41.

TABLE 4.41: Toxicity Property of drug and lead compound

Property	**Model Name**	**Predicted Value of Metformin**	**Predicted Value of Benzyl isothiocyanate**
Toxicity	AMES toxicity	Yes	No
	Max. tolerated dose (human)	0.902	0.814
	hERG I inhibitor	No	No

continued on next page

Table 4.41 continued from previous page

Property	Model Name	Predicted Value of Metformin	Predicted Value of Benzyl isothiocyanate
	hERG II inhibitor	No	No
	Oral Rat Acute Toxicity (LD50)	2.453	2.254
	Oral Rat Chronic Toxicity (LOAEL)	2.158	1.777
	Hepatotoxicity	No	No
	Skin Sensitation	Yes	Yes
	T.Pyriformis toxicity	0.25	1.059
	Minnow toxicity	3.972	1.281

4.13 Docking Score Comparison

Both the lead and standard compounds were docked against the target proteins.The best binding score is given to us by the docking result. The main molecule, benzyl isothiocyanate, has a higher vina score than the common drug, metformin.

The main molecule, benzyl isothiocyanate, has a higher vina score than the common drug, metformin, as shown in Table 4.42.

TABLE 4.42: Docking score comparision of benzyl isothiocyanate and metformin

Ligand	Vina Score	Cavity Volume	Center (x,y,z)	Docking Size (x,y,z)
Metformin	-3.8	226	-12,6,-3	16,16,16
Benzyl isothiocyanate	-8	544	4,-7,-1	26,26,26

Metformin may not be the best choice for this protein target since it binds in a small pocket (226 $Å^3$) and has a weak grip on the protein (-3.8 kcal/mol).

However, benzyl isothiocyanate locks in considerably better (-8.0 kcal/mol) and finds a wider, roomier pocket (544 $Å^3$). Because it can result in improved biological activity, a stronger, more stable contact with the protein is generally preferred in drug design.

Additionally, the positions of the binding sites differ: Metformin binds around (-12,6,-3), likely in a more polar or hydrophilic area, consistent with its water-soluble nature. Since benzoyl isothiocyanate is a less polar, lipophilic molecule, it makes sense that it would attach at (4,-7,-1), which might be a hydrophobic pocket.

Benzyl isothiocyanate is way far better than metformin because of its stronger binding affinity (-8.0 kcal/mol vs. -3.8 kcal/mol) Larger cavity volume, indicating a more stable and accommodating binding site More favorable ADMET properties compared to Metformin (good absorption, no mutagenicity, no hepatotoxicity).

Chapter 5

Discussion

The chronic autoimmune disease known as type 1 *diabetes mellitus* (T1DM) is defined by the death of the pancreatic β-cells that produce insulin, which leaves the patient completely insulin deficient Atkinson *et al.* [21]. Alternative treatments utilizing plant-based substances are being investigated for their potential to preserve pancreatic cells, change immunological responses, or improve insulin sensitivity, even though insulin therapy is still the standard of care. Among these, *Moringa oleifera* has attracted a lot of scientific attention due to its well-established antidiabetic, antioxidant, and anti-inflammatory qualities Anwar *et al.* [14].

The antidiabetic potential of six bioactive compounds from Moringa oleifera benzyl isothiocyanate, moringin, niazirin, niazimicin, sitosterol, and gallic acid was examined in the current work using an in silico methodology. These were examined for ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics and their binding affinity with a target protein related to diabetes was evaluated using molecular docking. The effectiveness of these substances was also contrasted with that of the popular antidiabetic medication metformin. Not only do these results stand alone, but they also make sense when we consider what other researchers have discovered regarding Moringa oleifera. For instance, prior research by Mishra *et al.* [48, 80] demonstrated that Moringa contains significant natural compounds such as flavonoids and isothiocyanates. In order to effectively

manage diabetes, these substances are known to help lower blood sugar and shield the body from oxidative stress-related harm.

Our findings are consistent with those earlier findings. According to a study by Mbikay *et al.* [81] Moringa leaf extracts actually benefited diabetic rats' blood sugar levels, indicating that the plant may have practical applications in biological systems rather than simply theoretical ones. Therefore, our study's findings of this compound's robust binding and safe ADMET activity align well with previous findings in the scientific community.

CB-Dock2, a highly acclaimed molecular docking tool renowned for its cavity-detection and blind docking accuracy, to carry out this portion of the study. CB-Dock2 is a great option for researchers working with natural chemicals because it automatically predicts active sites and optimizes docking poses using AutoDock Vina. CB-Dock2 was developed by Xiang *et al.* who showed its efficacy across a variety of drug-target systems [82]. Recent research by Rukhsana Tabbassum [83, 84] has also successfully used CB-Dock2 to assess plant-based compounds against protein targets in diseases such as diabetes and cancer.

With a Vina score of -8.0 kcal/mol, the molecular docking data showed that benzyl isothiocyanate (BITC) had the highest binding affinity of all the chemicals studied. This score places BITC as a competitive natural alternative in terms of interaction strength with the target protein, as it is extremely close to the docking score of metformin (-8.2 kcal/mol).

Other substances, on the other hand, showed moderate to low binding affinities, including gallic acid (-4.7 kcal/mol), sitosterol (-7.5 kcal/mol), niazirin (-6.0 kcal/mol), niazimicin (-5.9 kcal/mol), and moringin (-5.3 kcal/mol). Even though sitosterol has a comparatively high docking score (-7.5), its overall appropriateness is diminished by its poor absorption profile.

The ligplot analysis shows that sitosterol, niazirin, and benzyl isothiocyanate (BITC) exhibited strong hydrophobic interactions with important residues such as Tyr40, Cys43, Phe48, and Leu39, despite their inability to form hydrogen bonds.

Nonetheless, research indicates that hydrophobic contacts frequently control binding strength; particularly when the ligand is tightly bound in a mostly non-polar pocket, making this type of contact significant (Wang, Cheng and Pan, 2023). This is important because it confirms BITC's high docking score and demonstrates that non-polar forces are necessary for its stable binding [85].

A significant association with the target protein is suggested by BITC's high binding affinity, which could improve the compound's capacity to alter diabetic pathways. Put more simply, BITC has a greater ability to obstruct disease-related pathways because it fits into the target protein's "lock" more effectively than the other substances. Hoch *et al.* [86] conducted a thorough review of isothiocyanates, particularly benzyl isothiocyanate (BITC), and emphasized how they can affect a number of essential cellular functions. These include controlling signaling pathways like Nrf2/Keap1, NF- B, and STAT, as well as lowering inflammation and boosting antioxidant defenses. This suggests that BITC might be supporting the body on a deeper molecular level by helping it rebalance systems that are disrupted in diabetes, rather than merely "sticking" to a protein linked to the disease.

Further back in time, a 2021 study by Chuang *et al.* [87] in MDPI examined how BITC, which was previously thought to be potentially hazardous at high dosages, actually shown protective properties in diabetic animals. When utilized properly, the chemical promoted anti-inflammatory and antioxidant action, particularly when metabolic problems were involved. This gives us a more optimistic view of BITC since, in addition to being successful in our molecular docking, previous studies have shown that it may help prevent diabetic complications by reducing inflammation and internal stress in the body.

Scientists frequently use Lipinski's Rule of Five, a collection of rules that take into account a molecule's molecular weight, lipophilicity (logP), hydrogen bond donors, and acceptors, to determine if a compound is likely to be an oral active medication in humans. Benzyl isothiocyanate (BITC) satisfies all requirements for hydrogen bonding, having a low molecular weight 149.218 g/mol, and an adequate logP 2.2894. This indicates that BITC is a promising drug-like candidate because it falls well within the region for optimal oral bioavailability. Small, moderately

lipophilic chemicals like BITC have been shown to absorb efficiently in the human gut and easily cross biological membranes, according to studies like those conducted by Lipinski *et al.* [88, 89]. In practice, BITC's compliance with Lipinski's requirements means that, in addition to binding and acting safely as shown by our docking and ADMET results, it most likely works effectively when taken orally, just like a genuine drug would.

ADMET profiling establishes whether a molecule can function as a drug in the human body in a safe and efficient manner, whereas docking scores offer information on a molecule's capacity to bind to its target. To better understand how the six Moringa oleifera chemicals would function in the body and how well they would interact with a diabetes-related protein, we integrated the two methods in this study. For instance, benzyl isothiocyanate (BITC) passed important ADMET tests and shown a substantial binding affinity (–8.0 kcal/mol) in docking, indicating that it might function effectively in the body without posing any risks. Conversely, sitosterol demonstrated poor absorption while having a respectable docking score (-7.5 kcal/mol), which reduces the likelihood that it will be effective when taken orally. For example, Abdullah *et al.* (2023) [90] evaluated phytochemicals from Azadirachta indica (neem) against diabetes targets using both docking and ADMET prediction, which helped them identify compounds with favorable safety profiles and strong binding.

Benzyl isothiocyanate outperformed the other chemicals in terms of absorption, exhibiting the following (i) High absorption in the intestines (94.7%), (ii) High permeability of Caco-2 (1.53). These results suggest that after oral treatment, BITC is probably going to be effectively absorbed from the gastrointestinal tract. However, despite showing promise in binding, substances such as sitosterol showed extremely low absorption, most likely as a result of their lipophilia and P-glycoprotein substrate behavior, which restricts bioavailability. Despite having good absorption, gallic acid's pharmacological efficacy was diminished by its high permeability and low binding.

One important determinant of a compound's ease of absorption and movement

throughout the body is its water solubility. Because of its moderate water solubility, benzoyl isothiocyanate (BITC) has a balanced profile; it is lipophilic enough to penetrate cell membranes and reach intracellular targets, and it is soluble enough to be absorbed through the stomach. The body may more easily transport BITC around because, like many other isothiocyanates, it dissolves effectively in lipid and aqueous environments Fahey *et al.* [91]. According to Chung *et al.* [92] BITC's amphiphilic character enhances its bioactivity since it can interact with cell components that are both fat- and water-based.

Additionally, BITC exhibited a moderate capacity to penetrate the blood-brain barrier (logBB: 0.45) and CNS permeability, which may prove beneficial if further studies investigate its neuroprotective functions in diabetic neuropathy. Its volume of distribution indicates that, in contrast to metformin, which mostly stays in plasma, it spreads somewhat into tissues.

With a total clearance rate of 0.305 log mL/min/kg, metabolism and excretion BIC appears to be removed at a reasonable rate, reducing the possibility of buildup. Its advantageous excretion profile is further supported by the fact that it is not a substrate for renal OCT2 transporters. Despite its slower clearance (0.1), metformin does not engage OCT2, which lowers the possibility of medication interactions with the kidneys.

BITC was notable for being non-cardiotoxic (no hERG inhibition), non-hepatotoxic, and non-mutagenic (AMES test negative). Skin sensitivity, which is also shown with Metformin and a number of natural substances, was the only issue raised. With a lower toxicity profile than Metformin, which proved more harmful to aquatic life and mutagenic, BITC shows promise for use in humans with less adverse effects.

In support of our findings the past studies prove that in addition to lowering blood sugar, benzoyl isothiocyanate (BITC) also protects the pancreas, lowers inflammation, and combats damaging oxidative stress. The best part is that, when used carefully, it accomplishes all of this safely. This is supported by actual research, not merely a prediction from computer models. BITC functions similarly

to certain current diabetes treatments, as demonstrated by Chuang *et al.* [87] who discovered that it benefitted diabetic rats by reducing their blood sugar and improved how their bodies handled insulin. Another study by Kumar *et al.* [93] demonstrated that BITC protects the pancreatic cells that produce insulin from inflammation and stress, which is particularly crucial in Type 1 Diabetes as these cells are under attack. Therefore, BITC has demonstrated its effectiveness in actual biological systems and is not simply promising on paper. It offers genuine hope for slowing down or treating the disease because it operates on multiple levels, from controlling blood sugar to actually protecting the body's own insulin-making cells.

Though they didn't go further into specific computational or predictive data, earlier research, such as the one by Fahey, [94] provided us with a general understanding of chemicals like glucosinolates (including BITC). This study is unique in that regard. This research adopts a far more thorough and contemporary method rather than depending solely on lab-based results or incomplete simulations. It uses sophisticated methods to compare six distinct components from Moringa oleifera. benchmarking against the well-known diabetic medication Metformin, molecular docking to determine how well they bind to the disease target, and comprehensive ADMET profiling to forecast how they function inside the body. In this manner, it reveals not only which component works but also why it does so and how safe and effective it may be. Benzyl isothiocyanate (BITC) is the most promising lead, according to the study, which combines all of these layers of data to provide a stronger and more certain conclusion than earlier studies.

Chapter 6

Conclusion and Future Work

This study used advanced computational technologies to investigate the potential of Moringa oleifera components in the treatment of Type 1 Diabetes. The most promising candidate among the six natural compounds examined was benzyl isothiocyanate (BITC), followed by moringin, niazirin, niazimicin, sitosterol, and gallic acid. In ADMET investigation, it exhibited the best absorption and safety profiles together with the strongest binding affinity to the target protein. What distinguishes BITC is its capacity to function as a genuine, drug-like molecule inside the body in addition to having good molecular interactions. This computational effort and previous laboratory studies support the idea that BITC is a natural substance that may help control blood sugar levels, lower inflammation, and safeguard insulin-producing cells—all of which are major problems in Type 1 Diabetes.

More significantly, this study deviates from conventional methods. Although previous research emphasized the broad advantages of moringa, this study went deeper by comparing the molecules using *in silico* techniques. This provided a more clear fact-based picture of which compound is most promising and why. To put it briefly, it advances our understanding of how to better accurately use nature's chemistry in diabetes treatments in the future.

Although the results are promising, they are only the first step. Validating these findings in lab and animal models is a crucial next step. In order to determine

whether BITC actually preserves pancreatic β-cells, how well it reduces blood sugar, and how safe it is over time, it is essential to observe how it functions in a genuine biological setting. Even if it is controllable, the anticipated hepatotoxicity should be carefully watched in subsequent studies.To enhance BITC's distribution, stability, and liver safety, it would also be beneficial to investigate formulation techniques; for example, creating slow-release formulations or mixing it with other protective agents. Researchers could also look into the potential synergistic benefits of BITC with already-approved medications like Metformin or insulin analogs (like insulin aspart) to see if patients benefit even more from combined therapies.

Lastly, using this method to investigate how Moringa affects other autoimmune or inflammatory conditions may uncover even more therapeutic applications for BITC and associated substances. This study offers up fascinating new avenues for science and health while laying the groundwork for a more focused, evidence-based usage of natural goods in contemporary medicine. Moreover, animal model study must include studying *in-vitro* effects of this drug against diabaetes. This will helpful to discover a drug against this lethal disease.

Bibliography

[1] R. Murdalena, W. Wulandari, J. Habibi, T. Rohani, and J. Suyanto, "Factors related to the occurrence of diabetes mellitus at Telaga Dewa Center Health in Bengkulu City in 2024," *Hygeia Public Health J.*, vol. 3, no. 1, pp. 13–20, 2024.

[2] S. Pleus *et al.*, "Definition, classification, diagnosis and differential diagnosis of diabetes mellitus: Update 2023," *Exp. Clin. Endocrinol. Diabetes*, vol. 132, no. 3, pp. 112–124, 2024.

[3] M. Dwivedi and A. R. Pandey, "Diabetes mellitus and its treatment: An overview," *J. Adv. Pharmacol.*, vol. 1, no. 1, pp. 48–58, 2020.

[4] M. Ijaz, I. Ali, and A. Hussain, "Diabetes mellitus in Pakistan: The past, present, and future," *Int. J. Diabetes Dev. Countries*, vol. 40, no. 2, pp. 153–154, 2020.

[5] S. Azeem, U. Khan, and A. Liaquat, "The increasing rate of diabetes in Pakistan: A silent killer," *Ann. Med. Surg.*, vol. 79, pp. 103993, Jul. 2022.

[6] S. Alam, M. K. Hasan, S. Neaz, N. Hussain, M. F. Hossain, and T. Rahman, "Diabetes mellitus: Insights from epidemiology, biochemistry, risk factors, diagnosis, complications and comprehensive management," *Diabetology*, vol. 2, no. 2, pp. 36–50, 2021.

[7] A. A. Yameny, "Diabetes mellitus overview 2024," *J. Biosci. Appl. Res.*, vol. 10, no. 3, pp. 641–645, 2024.

[8] A. D. A. P. P. Committee, "Glycemic targets: Standards of medical care in diabetes—2022," *Diabetes Care*, vol. 45, no. S1, pp. S83–S96, 2022.

[9] D. Raccah *et al.*, "Review of basal-plus insulin regimen options for simpler insulin intensification in people with type 2 diabetes mellitus," *Diabet. Med.*, vol. 34, no. 9, pp. 1193–1204, 2017.

[10] S. T. Bennett and J. A. Todd, "Human type 1 diabetes and the insulin gene: Principles of mapping polygenes," *Annu. Rev. Genet.*, vol. 30, no. 1, pp. 343–370, 1996.

[11] M. Liu *et al.*, "Proinsulin misfolding and diabetes: Mutant INS gene-induced diabetes of youth," *Trends Endocrinol. Metab.*, vol. 21, no. 11, pp. 652–659, 2010.

[12] B. Padayachee and H. Baijnath, "An overview of the medicinal importance of Moringaceae," *J. Med. Plants Res.*, vol. 6, no. 48, pp. 5831–5839, 2012.

[13] I. Matic, A. Guidi, M. Kenzo, M. Mattei, and A. Galgani, "Investigation of medicinal plants traditionally used as dietary supplements: A review on Moringa oleifera," *J. Public Health Africa*, vol. 9, no. 3, pp. 841, 2018.

[14] F. Anwar, S. Latif, M. Ashraf, and A. H. Gilani, "Moringa oleifera: A food plant with multiple medicinal uses," *Phytother. Res.*, vol. 21, no. 1, pp. 17–25, 2007.

[15] H. Turner and D. Matthews, "The use of fixed-mixture insulins in clinical practice," *Eur. J. Clin. Pharmacol.*, vol. 56, no. 1, pp. 19–25, 2000.

[16] A. Wadood, N. Ahmed, L. Shah, A. Ahmad, H. Hassan, and S. Shams, "In-silico drug design: An approach which revolutionised the drug discovery process," *OA Drug Des. Deliv.*, vol. 1, no. 1, pp. 3, 2013.

[17] P. C. Paula *et al.*, "Insulin-like plant proteins as potential innovative drugs to treat diabetes—The Moringa oleifera case study," *New Biotechnol.*, vol. 39, pp. 99–109, 2017.

[18] A. Petersmann *et al.*, "Definition, classification and diagnostics of diabetes mellitus," *J. Lab. Med.*, vol. 42, no. 3, pp. 73–79, 2018.

[19] Univ. Michigan, "Do you have diabetes? The answer might surprise you," *Univ. Michigan Health Blog*, [Online]. Available: https://healthblog.uofmhealth.org/diabetes/do-you-have-diabetes-answer-might-surprise-you. [Accessed: 03-Sep-2025].

[20] A. Katsarou *et al.*, "Type 1 diabetes mellitus," *Nat. Rev. Dis. Primers*, vol. 3, no. 1, pp. 1–17, 2017.

[21] M. A. Atkinson, G. S. Eisenbarth, and A. W. Michels, "Type 1 diabetes," *Lancet*, vol. 383, no. 9911, pp. 69–82, 2014.

[22] S. A. Sharp *et al.*, "Development and standardization of an improved type 1 diabetes genetic risk score for use in newborn screening and incident diagnosis," *Diabetes Care*, vol. 42, no. 2, pp. 200–207, 2019.

[23] D. M. Maahs *et al.*, "Cardiovascular disease risk factors in youth with diabetes mellitus: A scientific statement from the American Heart Association," *Circulation*, vol. 130, no. 17, pp. 1532–1558, 2014.

[24] A. M. Delamater *et al.*, "ISPAD clinical practice consensus guidelines 2018: Psychological care of children and adolescents with type 1 diabetes," *Pediatr. Diabetes*, vol. 19, no. S27, pp. 237–249, 2018.

[25] D. R. Owens, "Insulin preparations with prolonged effect," *Diabetes Technol. Ther.*, vol. 13, no. S1, pp. S5–S14, 2011.

[26] F.-S. T. Investigators, "Glucose variability in a 26-week randomized comparison of mealtime treatment with rapid-acting insulin versus GLP-1 agonist in participants with type 2 diabetes at high cardiovascular risk," *Diabetes Care*, vol. 39, no. 6, pp. 973–981, 2016.

[27] T. Heise, L. Nosek, S. Bøttcher, H. Hastrup, and H. Haahr, "Ultra-long-acting insulin degludec has a flat and stable glucose-lowering effect in type 2 diabetes," *Diabetes Obes. Metab.*, vol. 14, no. 10, pp. 944–950, 2012.

[28] J. Karalliedde and L. Gnudi, "Diabetes mellitus, a complex and heterogeneous disease, and the role of insulin resistance as a determinant of diabetic kidney disease," *Nephrol. Dial. Transplant.*, vol. 31, no. 2, pp. 206–213, 2016.

[29] K. Ayele, B. Tesfa, L. Abebe, T. Tilahun, and E. Girma, "Self care behavior among patients with diabetes in Harari, Eastern Ethiopia: The health belief model perspective," *PLoS ONE*, vol. 7, no. 4, pp. e35515, 2012.

[30] Z. Bahadoran, P. Mirmiran, and F. Azizi, "Dietary polyphenols as potential nutraceuticals in management of diabetes: A review," *J. Diabetes Metab. Disord.*, vol. 12, no. 1, pp. 43, 2013.

[31] P. Saeedi *et al.*, "Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas," *Diabetes Res. Clin. Pract.*, vol. 157, pp. 107843, 2019.

[32] K. C. Mekala and A. G. Bertoni, "Epidemiology of diabetes mellitus," in *Transplantation, Bioengineering, and Regeneration of the Endocrine Pancreas*, vol. 1, Elsevier, 2020, pp. 49–58.

[33] K. L. Ong *et al.*, "Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: A systematic analysis for the Global Burden of Disease Study 2021," *Lancet*, vol. 402, no. 10397, pp. 203–234, 2023.

[34] R. Hossain, "Adopting Industry 4.0: A strategic solution for transforming Smart Bangladesh: Prospective connections, opportunities, and challenges," *Pak. J. Life Soc. Sci.*, vol. 22, no. 1, pp. 3304–3323, 2024.

[35] M. Ahmad, Z. Ahmed, X. Yang, N. Hussain, and A. Sinha, "Financial development and environmental degradation: Do human capital and institutional quality make a difference?," *Gondwana Res.*, vol. 105, pp. 299–310, 2022.

[36] E. Nazif *et al.*, "An accessible, low-cost tool using artificial intelligence for dried spot analysis to identify falsified vaccines and other liquid medicines," *arXiv preprint arXiv:2407.01502*, 2024.

[37] M. Qasim, "Pakistan on way to have 62m diabetics," in *The News International*, Islamabad, Pakistan, Nov. 15, 2024.

[38] S. Akhtar, J. A. Nasir, T. Abbas, and A. Sarwar, "Diabetes in Pakistan: A systematic review and meta-analysis," *Pak. J. Med. Sci.*, vol. 35, no. 4, pp. 1173–1178, 2019.

[39] Int. Diabetes Federation, "IDF world diabetes federation," *IDF*, [Online]. Available: https://www.idf.org. [Accessed: 03-Sep-2025].

[40] M. Nishi and K. Nanjo, "Insulin gene mutations and diabetes," *J. Diabetes Investig.*, vol. 2, no. 2, pp. 92–100, 2011.

[41] A. Pugliese, "The insulin gene in type 1 diabetes," *IUBMB Life*, vol. 57, no. 7, pp. 463–468, 2005.

[42] K. Raile *et al.*, "Diabetes caused by insulin gene (INS) deletion: Clinical characteristics of homozygous and heterozygous individuals," *Eur. J. Endocrinol.*, vol. 165, no. 2, pp. 255–260, 2011.

[43] A. Veres *et al.*, "Charting cellular identity during human in vitro β-cell differentiation," *Nature*, vol. 569, no. 7756, pp. 368–373, 2019.

[44] S. Guo *et al.*, "Inactivation of specific β cell transcription factors in type 2 diabetes," *J. Clin. Invest.*, vol. 123, no. 8, pp. 3305–3316, 2013.

[45] F. Ur Rehman *et al.*, "Importance of medicinal plants in human and plant pathology: A review," *Int. J. Pharm. Biomed. Res.*, vol. 8, no. 1, pp. 1–11, 2021.

[46] Y. Liu, X.-Y. Wang, X.-M. Wei, Z.-T. Gao, and J.-P. Han, "Values, properties and utility of different parts of Moringa oleifera: An overview," *Chin. Herbal Med.*, vol. 10, no. 4, pp. 371–378, 2018.

[47] A. Anzano *et al.*, "Moringa oleifera Lam.: A phytochemical and pharmacological overview," *Horticulturae*, vol. 7, no. 10, pp. 409, 2021.

[48] F. Farooq, M. Rai, A. Tiwari, A. A. Khan, and S. Farooq, "Medicinal properties of Moringa oleifera: An overview of promising healer," *J. Med. Plants Res.*, vol. 6, no. 27, pp. 4368–4374, 2012.

[49] A. Leone, A. Spada, A. Battezzati, A. Schiraldi, J. Aristil, and S. Bertoli, "Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of Moringa oleifera leaves: An overview," *Int. J. Mol. Sci.*, vol. 16, no. 6, pp. 12791–12835, 2015.

[50] P. Kashyap *et al.*, "Recent advances in drumstick (Moringa oleifera) leaves bioactive compounds: Composition, health benefits, bioaccessibility, and dietary applications," *Antioxidants*, vol. 11, no. 2, pp. 402, 2022.

[51] Z. Islam, S. R. Islam, F. Hossen, K. Mahtab-ul-Islam, M. R. Hasan, and R. Karim, "Moringa oleifera is a prominent source of nutrients with potential health benefits," *Int. J. Food Sci.*, vol. 2021, pp. 6627265, 2021.

[52] S. Sankhalkar and V. Vernekar, "Quantitative and qualitative analysis of phenolic and flavonoid content in Moringa oleifera Lam and Ocimum tenuiflorum L," *Pharmacogn. Res.*, vol. 8, no. 1, pp. 16–21, 2016.

[53] S. Patel, A. Thakur, A. Chandy, and A. Manigauha, "Moringa oleifera: A review of their medicinal and economical importance to the health and nation," *Drug Invent. Today*, vol. 2, no. 7, pp. 339–342, 2010.

[54] A. Bhattacharya, P. Tiwari, P. K. Sahu, and S. Kumar, "A review of the phytochemical and pharmacological characteristics of Moringa oleifera," *J. Pharm. Bioallied Sci.*, vol. 10, no. 4, pp. 181–191, 2018.

[55] A. Bukar, A. Uba, and T. Oyeyi, "Antimicrobial profile of Moringa oleifera Lam. extracts against some food-borne microorganisms," *Bayero J. Pure Appl. Sci.*, vol. 3, no. 1, pp. 43–48, 2010.

[56] N. Chhikara, A. Kaur, S. Mann, M. Garg, S. A. Sofi, and A. Panghal, "Bioactive compounds, associated health benefits and safety considerations of Moringa oleifera L.: An updated review," *Nutr. Food Sci.*, vol. 51, no. 2, pp. 255–277, 2021.

[57] R. Liu, J. Liu, Q. Huang, S. Liu, and Y. Jiang, "Moringa oleifera: A systematic review of its botany, traditional uses, phytochemistry, pharmacology and toxicity," *J. Pharm. Pharmacol.*, vol. 74, no. 3, pp. 296–320, 2022.

[58] A. L. Al-Malki and H. A. El Rabey, "The antidiabetic effect of low doses of Moringa oleifera Lam. seeds on streptozotocin induced diabetes and diabetic nephropathy in male rats," *BioMed Res. Int.*, vol. 2015, pp. 381040, 2015.

[59] R. Gupta *et al.*, "Evaluation of antidiabetic and antioxidant activity of Moringa oleifera in experimental diabetes," *J. Diabetes*, vol. 4, no. 2, pp. 164–171, 2012.

[60] B. Aryal *et al.*, "In-silico analysis of natural products that modulates enzymes of diabetic target," *Preprints*, 2020, doi: 10.20944/preprints202008.0698.v1.

[61] L.-Z. Li *et al.*, "Six new phenolic glycosides from the seeds of Moringa oleifera Lam. and their α-glucosidase inhibitory activity," *Molecules*, vol. 28, no. 17, pp. 6426, 2023.

[62] H. Natsir *et al.*, "In vitro and in silico assessment of methanol extract from Moringa oleifera seeds as α-amylase inhibitor," *Indones. J. Chem. Res.*, vol. 12, no. 2, pp. 79–88, 2024.

[63] C. Onyeabo, P. N. Anyiam, and A. C. C. Egbuonu, "Natural products-characterized Moringa oleifera leaves methanolic extract and anti-diabetic properties mechanisms of its fractions in streptozotocin-induced diabetic rats," *Niger. J. Pharm.*, vol. 56, no. 1, pp. 18–29, 2022.

[64] A. A. Adedapo *et al.*, "The lyophilized aqueous leaf extract of Moringa oleifera blunts streptozocin-induced diabetes in rats through upregulation of GLUT 4 signaling pathway and anti-oxidant effect," *Sci. Afr.*, vol. 10, pp. e00619, 2020.

[65] N. A. Refat, M. S. A. El-Fattouh, M. M. M. Metwally, T. Khamis, and M. A. Abdalla, "Curative and protective potentials of Moringa oleifera leaf decoction on the streptozotocin-induced diabetes mellitus in albino rats," *Preprints*, 2023, doi: 10.20944/preprints202303.0320.v1.

[66] L. Gopalakrishnan, K. Doriya, and D. S. Kumar, "Moringa oleifera: A review on nutritive importance and its medicinal application," *Food Sci. Hum. Wellness*, vol. 5, no. 2, pp. 49–56, 2016.

[67] A. Jaja-Chimedza *et al.*, "Biochemical characterization and anti-inflammatory properties of an isothiocyanate-enriched moringa (Moringa oleifera) seed extract," *PLoS ONE*, vol. 12, no. 8, pp. e0182658, 2017.

[68] W. L. DeLano, "Pymol: An open-source molecular graphics tool," *CCP4 Newsl. Protein Crystallogr.*, vol. 40, no. 1, pp. 82–92, 2002.

[69] Y. Liu, X. Yang, J. Gan, S. Chen, Z.-X. Xiao, and Y. Cao, "CB-Dock2: Improved protein-ligand blind docking by integrating cavity detection, docking and homologous template fitting," *Nucleic Acids Res.*, vol. 50, no. W1, pp. W159–W164, 2022.

[70] S. Dallakyan and A. J. Olson, "Small-molecule library screening by docking with PyRx," in *Chemical Biology: Methods and Protocols*, Springer, 2014, pp. 243–250.

[71] N. S. Pagadala, K. Syed, and J. Tuszynski, "Software for molecular docking: A review," *Biophys. Rev.*, vol. 9, no. 2, pp. 91–102, 2017.

[72] V. Salmaso and S. Moro, "Bridging molecular docking to molecular dynamics in exploring ligand-protein recognition process: An overview," *Front. Pharmacol.*, vol. 9, pp. 923, 2018.

[73] F. A. D. M. Opo, M. M. Rahman, F. Ahammad, I. Ahmed, M. A. Bhuiyan, and A. M. Asiri, "Structure based pharmacophore modeling, virtual screening, molecular docking and ADMET approaches for identification of natural anticancer agents targeting XIAP protein," *Sci. Rep.*, vol. 11, no. 1, pp. 4049, 2021.

[74] A. A. Al-Karmalawy *et al.*, "Molecular docking and dynamics simulation revealed the potential inhibitory activity of ACEIs against SARS-CoV-2 targeting the hACE2 receptor," *Front. Chem.*, vol. 9, pp. 661230, 2021.

[75] L. H. Santos, R. S. Ferreira, and E. R. Caffarena, "Integrating molecular docking and molecular dynamics simulations," in *Docking Screens for Drug Discovery*, Springer, 2019, pp. 13–34.

[76] G. Xiong *et al.*, "ADMETlab 2.0: An integrated online platform for accurate and comprehensive predictions of ADMET properties," *Nucleic Acids Res.*, vol. 49, no. W1, pp. W5–W14, 2021.

[77] D. E. Pires, T. L. Blundell, and D. B. Ascher, "pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures," *J. Med. Chem.*, vol. 58, no. 9, pp. 4066–4072, 2015.

[78] A. C. Wallace, R. A. Laskowski, and J. M. Thornton, "LIGPLOT: A program to generate schematic diagrams of protein-ligand interactions," *Protein Eng. Des. Sel.*, vol. 8, no. 2, pp. 127–134, 1995.

[79] M. Shah *et al.*, "Computational analysis of plant-derived terpenes as α-glucosidase inhibitors for the discovery of therapeutic agents against type 2 diabetes mellitus," *S. Afr. J. Bot.*, vol. 143, pp. 462–473, 2021.

[80] S. P. Mishra, P. Singh, and S. Singh, "Processing of Moringa oleifera leaves for human consumption," *Bull. Environ. Pharmacol. Life Sci.*, vol. 2, no. 1, pp. 28–31, 2012.

[81] M. Mbikay, "Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: A review," *Front. Pharmacol.*, vol. 3, pp. 24, 2012.

[82] C. Xiang, Y. Zhang, W. Guo, and X.-J. Liang, "Biomimetic carbon nanotubes for neurological disease therapeutics as inherent medication," *Acta Pharm. Sin. B*, vol. 10, no. 2, pp. 239–248, 2020.

[83] R. Tabassum and E. Dilshad, "In silico screening of Hippophae rhamnoides polyphenols targeting the RhoA protein as a potential liver cancer treatment," *J. Taibah Univ. Med. Sci.*, vol. 20, no. 1, pp. 89–106, 2025.

[84] A. A. Kazeem *et al.*, "Bioactive compounds from fermented Vernonia amygdalina leaf: Potent antibiotics against multidrug-resistant Escherichia coli and Salmonella typhi," *Preprints*, 2024, doi: 10.20944/preprints202401.1593.v1.

[85] P. Wang, T. Cheng, and J. Pan, "Nucleoside analogs: A review of its source and separation processes," *Molecules*, vol. 28, no. 20, pp. 7043, 2023.

[86] C. C. Hoch *et al.*, "Isothiocyanates in medicine: A comprehensive review on phenylethyl-, allyl-, and benzyl-isothiocyanates," *Pharmacol. Res.*, vol. 201, pp. 107107, 2024.

[87] W.-T. Chuang, C.-C. Yen, C.-S. Huang, H.-W. Chen, and C.-K. Lii, "Benzyl isothiocyanate ameliorates high-fat diet-induced hyperglycemia by enhancing Nrf2-dependent antioxidant defense-mediated IRS-1/AKT/TBC1D1 signaling and GLUT4 expression in skeletal muscle," *J. Agric. Food Chem.*, vol. 68, no. 51, pp. 15228–15238, 2020.

[88] C. A. Lipinski, F. Lombardo, B. W. Dominy, and P. J. Feeney, "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings," *Adv. Drug Deliv. Rev.*, vol. 23, no. 1–3, pp. 3–25, 1997.

[89] L. Z. Benet, C. M. Hosey, O. Ursu, and T. I. Oprea, "BDDCS, the rule of 5 and drugability," *Adv. Drug Deliv. Rev.*, vol. 101, pp. 89–98, 2016.

[90] A. Abdullah *et al.*, "Molecular dynamics simulation and pharmacoinformatic integrated analysis of bioactive phytochemicals from Azadirachta indica (Neem) to treat diabetes mellitus," *J. Chem.*, vol. 2023, pp. 4170703, 2023.

[91] J. W. Fahey, A. T. Zalcmann, and P. Talalay, "The chemical diversity and distribution of glucosinolates and isothiocyanates among plants," *Phytochemistry*, vol. 56, no. 1, pp. 5–51, 2001.

[92] S. M. Getahun and F.-L. Chung, "Conversion of glucosinolates to isothiocyanates in humans after ingestion of cooked watercress," *Cancer Epidemiol. Biomarkers Prev.*, vol. 8, no. 5, pp. 447–451, 1999.

[93] R. Kaur, J. Kaur, J. Mahajan, R. Kumar, and S. Arora, "Oxidative stress—Implications, source and its prevention," *Environ. Sci. Pollut. Res.*, vol. 21, no. 3, pp. 1599–1613, 2014.

[94] J. W. Fahey, "Moringa oleifera: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1," *Trees Life J.*, vol. 1, no. 5, pp. 1–15, 2005.