**CAPITAL UNIVERSITY OF SCIENCE AND
TECHNOLOGY, ISLAMABAD**

Identification of Anti inflammatory Metabolites From *Trigonella foenum graecum*

by

Muhammad Maaz

A thesis submitted in partial fulfillment for the
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in the
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*Dedicated to **ALLAH Almighty**, **Hazrat Muhammad (PBUH)**, my Parents and my respected Teachers who have been a constant source of motivation and encouragement during the challenges and supporting me spiritually throughout my life.*

CERTIFICATE OF APPROVAL

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Abstract

Current lifestyles, stress and toxic waste have extremely increased the incidence of various diseases in humans. Worldwide, researchers are looking for therapeutic agents that can cure or delay the onset of diseases. In the advanced countries, herbal drugs are in high demand for primary health care due to their efficacy, safety and low side effects as compare to synthetic medicine. So there are many herbal remedies that are used today to treat various infectious diseases. *Trigonella foenum-graecum* is an annual plant with in the family Fabaceae. This plant is used as a spice throughout the world and commonly grown in Pakistan, India and some Middle Eastern countries, which has many beneficial medicinal effects. It is known for its medicinal properties for example anti-inflammatory, anticarcinogenic, antidiabetic and immunological activities. *Trigonella foenum-graecum* edible part of the leaves contains moisture (86.1%), carbohydrates (6%), protein (4.4%), minerals (1.5%), fiber (1.1%) and fat (0.9%). Most important phytochemicals found in fenugreek seeds are carbohydrates, proteins, lipids, alkaloids, flavonoids, fiber and steroidal saponins, etc., which can be classified as volatile and non-volatile ingredients. Hence these plants are rich source of bioactive compounds. Identifying natural, plant-based and non-toxic anti-inflammatory drugs is essential for the treatment of various inflammatory diseases. Cyclooxygenas-2, mPGES-2, HNE and tyrosinase were selected as the target proteins and alkaloid, flavonoid, phytic acid, 4-hydroxyisoleucin, sapogenin, quercetin, trigonelline, tricin, naringenin and flavonol were selected as a ligands for the current study. Molecular Docking is technique used to estimate the strength of a bond between a ligand and a target protein through a special scoring function and to determine the correct structure of the ligand within the target binding site. The 3D structure of the target proteins and the ligands was taken as the input for docking. The docking was performed using Cox-2, m PGES-2, HNE and Tyrosinase proteins and ligands were alkaloid, flavonoid, phytic acid, 4-hydroxyisoleucin, sapogenin, quercetin, trigonelline, tricin, naringenin and flavonol. The best ligand was selected on the basis of best docking score, logp value, hydrogen bond acceptor, hydrogen bond donor and molecular weight. Sapogenin was identified as the best lead compound which shows the best

docking score, hydrogen bonding and pharmacokinetic properties as compare to other ligands such as alkaloid, flavonoid, phytic acid, 4-hydroxyisoleucin, quercetin, trigonelline, tricin, naringenin and flavonol. The selection of most efficient anti-inflammatory drug was based on the physiochemical and ADMET properties along mechanism of action with side effects. Therefore celebrex was selected as a best anti-inflammatory drug as compare to other anti-inflammatory drugs such as Aspirin, Ibuprofen, Paracetamol, Diclofenac and Naproxen. The comparison between Celebrex and Sapogenin help us to identify the better treatment for infectious diseases. Comparison was being performed through parameters like; ADMET properties and physiochemical properties. So it is determine that Sapogenins bioactive compound which shows us better result over celebrex according to comparison. All the software's and tools used in the current research study are reliable and authenticate.

Keywords: Medicinal Plants, *Trigonella Foenum Graecum*, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), Molecular Docking, Proteins, Ligands, ADMET Properties, Physiochemical Properties.

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Abbreviations

ADMET	Absorption Distribution Metabolism Excretion & Toxicity
AI	Aliphatic Index
BBB	Blood brain barrier
Cox-2	Cyclooxygenase-2
CB Dock	Cavity-detection guided Blind Docking
CADD	Computer-Aided Drug Designing
CNS	Central Nervous System
CYP2D6	Cytochrome P450 2D6
FDA	Food and Drug Administration
GRAVY	Grand average of hydropathicity
HNE	Human neutrophil elastase
HBA	Hydrogen Bond Acceptor
HBD	Hydrogen Bond Donor
hERG	Human Ether-a-go-go-Related Gene
II	Instability Index
KEGG	Kyoto Encyclopedia of genes and genomes
mPGES-2	Microsomal prostaglandin E synthase-2
MW	Molecular weight
NR	Total number of negatively charged residues (Asp + Glu)
NSAIDs	Non Steroidal Anti-inflammatory Drugs
OCT2	Organic cation transporter 2
PTGS	Prostaglandins
PGE2	Prostaglandin-E2
PDB	Protein data bank

pI   Theoretical pI

PR   Total number of positively charged residues (Asp + Glu)

TYRP-1   Tyrosinase Protein 1

TYRP-2   Tyrosinase Protein 2

VDss   Volume of Distribution at steady state

WHO   World Health Organization

Chapter 1

Introduction

1.1 Background

Genus Trigonella foenum-graecum (fenugreek) is an annual plant within the family Fabaceae. Fenugreek also called methi in Hindi, is an aromatic herbaceous plant and is a very significant spice and is commonly used as a traditional food and medicine. The dried seeds of these plants are extremely used in food and beverages as a flavor and supplement [1]. It is grown all over the world as a dry crop. Its seeds and leaves are common ingredients in transportation from the Indian subcontinent and are mostly used in traditional medicine. The *Trigonella foenum-graecum* edible part of the leaves contains moisture, carbohydrates, protein, minerals, fiber and fat. Hence these plants are good source of proteins, fats, carbohydrates, minerals and vitamins for example vitamin A, B1 and C. *T. foenum-graecum* when added to food, have antimicrobial activity to stop the growth of microorganisms in food. *Trigonella foenum-graecum* has antibacterial, antifungal, and antioxidant properties and therefore they can successfully stop the growth of microbial pathogens and help in food preservation and improve the shelf life of foods [2].

The seed and leaves of this plant mostly are used for the treatment of many other diseases in several traditional systems together with Ayurvedic medicine.

In Ayurvedic system, individually fenugreek seeds and leaves are used to prepare powder or extract for medicinal purposes [3]. *Trigonella foenum-graecum* cold water extract, called fenugreek tea, is usually used against respiratory infections (pneumonia & bronchitis) and subsequently it nourishes the body during illness, this herb is specifically used to reduce temperature, inflammation when taken with honey and lemon. This plant has been shown to possess, hypolipidemic and hypoglycaemic activities [4]. The plant's fenugreek is known for its ability to maintain cholesterol levels, as well as its ability to maintain glucose levels. *Trigonella foenum-graecum* pharmacological properties as well as antiviral, antimicrobial, antioxidant, anti-inflammatory activity have been reported [5]. The pharmacological and biological actions of fenugreek are recognized due to the diversity of its constituents as well as secondary metabolites which is derived additionally called phytochemicals. Most important phytochemicals present in fenugreek seeds are carbohydrates, proteins, lipids, alkaloids, flavonoids, fiber and steroidal saponins, etc., which can be classified as volatile and non-volatile ingredients [6].

Inflammation is defense mechanisms that protect tissue from harmful stimuli such as pathogens, allergens and damaged cells. This is often usually characterized by redness, swelling, pain and heat at the affected site. The mechanisms concerned within the inflammation like release of amine, bradykinin, and prostaglandins. When the inflammatory pathway is activated, the cells release a number of pro-inflammatory factors, containing several cytokines and chemokine's, to protect against disease causing agents such as pathogen. It is recognized that inflammation can release molecules, which create micro-environments that can be extremely helpful [7].

Cyclooxygenase enzyme also called prostaglandins (PTGS), which is responsible for the formation of prostanoids, together with arachidonic acid from prostaglandins such as Thromboxane and Prostaglandin. There are two isoforms of cyclooxygenase that are known as cyclooxygenase-1 and Cyclooxygenase-2. Cyclooxygenase-1 functions as a housekeeping isoform of Cyclooxygenases and is mainly expressed to serve functions resembling management of urinary organ blood flow, stomach protection against ulcers and preparation of prostaglandin E-2 [8]. On the other

hand Cyclooxygenase- 2 is associated in an inducible initial response which is activated in response to genes and numerous extracellular or intracellular physiological stimuli. Cyclooxygenase-2 is an important mediator of the inflammatory pathway [9]. Associated protein cyclooxygenase (COX-2) could also be responsible for the high levels of prostaglandins (PGs) in abundant inflammatory condition. Cyclooxygenase-2 excessive expression metabolizes accumulation of PGE2. Cyclooxygenase-2 is a selective inhibitor that can be a category of non-steroidal anti-inflammatory drugs (NSAIDs) which directly targeted cyclooxygenase-2 [10].

The molecules that targeted the flow of prostaglandin synthesis-2 regulate many signaling pathways and control apoptotic proteins and thus contribute to numerous physiological processes as well as proliferation, survival, mutation and metastasis. And therefore the excessive-expression and up-regulation of cyclooxygenase-2 are mostly connected with inflammation, unchecked cell division and cell death. Fenugreek seeds were found to possess anti-inflammatory activities. The inflammatory action of fenugreek is due to the inhibition of cyclooxygenase proteins (COX-1 and COX-2) and lipid peroxidation due to the inhibition of flavonoids-C-glycosides, triglycerides and fatty acids present inside the seeds. [11].

Prostaglandin E2 (PGE2) is an eicosanoid lipid mediator that significantly contributes to the pathogenesis of many inflammatory diseases. PGE-2 is formed when phospholipase secreted arachidonic acid from the plasma membrane and contains two cyclooxygenases such as cyclooxygenase-1 and cyclooxygenase-2 and specific isomers, like mPGES-1 and mPGES-2. The most commonly used nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, silicoxyb and acetaminophen (Paracetamol) have been shown to block inflammation, pain, and fever by blocking cyclooxygenase activity and thus reducing the generation of PGE-2 in injured tissues and proven useful in the treatment of Nervous system [12]. Consequently, the flow pathway elements of cyclooxygenase enzymes in the biosynthetic reaction of PGE-2, terminal synthesis and receptors are considered more specific targets for the treatment of pain and inflammation. Out of three-terminal synthesis, mPGES-1 frequently combines functionally with COX-2 and is the primary source of inflammatory prostaglandin E- 2 synthesis. Inhibition of

mPGES-1 has shown similar effects with NSAIDs in various experimental animal models. That's why, the development of mPGES-1 inhibitors is still complex. Our studies suggested that, while in treatment strategies targeting macrophages have to be challenged, macrophages serve as a better drug target for mPGES-1 anti-inflammatory therapy that could potentially As COX-2 can avoid inhibition. However, such an approach would actually protect the analgesic efficacy expected from NSAIDs, which needs further verification [13]. Prostaglandin E synthase-2 combines with four specific G proteins to act on receptor subtypes, called EP1–4 receptor. Targeting four receptors which are also being followed in the development of analgesics as an alternative approach to CoX-2 inhibitors. Targeting mPGES-1 and possibly four PGE-2 receptors could serve as promising strategies for the evolution of anti-inflammatory drugs for the next generation [14].

Hyperpigmentation or Hypopigmentation of the skin after infection is a very common sign. Several acute or chronic inflammatory skin reactions can produce a change in skin color. Melanosome are unique organs to be found in the cytoplasm of melanocytes, containing important enzymes that control the pigmentation of skin color, such as tyrosinase enzymes and its type's TYRP-1 and TYRP-2 [15]. SO current studies have confirmed that interleukin IL-1, IL-4, IL-6 and other inflammatory mediators can directly or indirectly control the proliferation and differentiation of human melanocytes and melanogenesis [16]. Neutrophils elastase is another enzyme which plays a vital part in the pathogenesis of many infections, from chronic inflammatory diseases to infectious diseases [17]. Human neutrophil elastase (HNE) is a protease enzyme that belongs to the chemotrypsin-like family of serine proteins. Neutrophils survive quickly in the blood of healthy people (round about 8 hours), but after inflammation they become active and their longevity can increase up to 5 days. When inflammatory stimuli develop in peripheral tissues, neutrophils are quickly recruited to the anatomical location of inflammation. Neutrophil degranulation is responsible for the release of various inflammatory mediators for example neutrophil elastase, catechin G and proteinase-3 [18]. 4-hydroxyisoleucine, alkaloid, flavonoid and phytic acid are the derivatives of *T.foenum-graecum.* The alkaloid and flavonoid content of fenugreek

seeds might be responsible for the inflammatory effects. 4-hydroxyisoleucine could be a unique organic compound isolated from fenugreek seeds. Phytic acid is abundant in nature (especially in plants). It accounts for 1to 5% weight of edible cereals, legumes, nuts, oil seeds and these are also derived from fenugreek [19].

Docking is an Insilco method used to estimate the strength of a bond between a ligand and a target protein through a special scoring function and to determine the correct structure of the ligand within the target binding site. The 3Dimentional structure of the target proteins and the ligands is taken as the input for docking [20]. It is recognized that this novel small molecular compounds, demonstrates essential properties, such as high interaction between target binding to target proteins with proper absorption, distribution, metabolism and excretion (ADME) to help in lead selection for the target [21]. The purpose of molecular docking is to simulate the process of molecular identification of a target protein and the ligands. It also focuses on achieving the minimum independent energy of the whole system, which includes proteins and ligands with proper alignment [22]. The mechanism of molecular docking of the proteins can be performed between small ligands, protein peptides, protein proteins, and protein nucleotides. Most frequent software used for docking are Auto Dock vina, Auto Dock, CB Dock and ICM etc [23]. Therefore various bioactive compounds present in fenugreek have been reported to possess a diversity of pharmacological and biological activities for example; antibacterial, antidiabetic, anti-inflammatory, anticarcinogenic and antioxidant activities. So thus all of these derivatives have been considered as a ligand and all of them having potential to dock with different enzymes such as microsomal prostaglandin E synthase-2 (mPGES-2), cyclooxygenase-2 (COX-2), human neutrophil elastase (HNE) and tyrosinase enzymes [24].

1.2 Problem Statement

Probably it is estimated that the start of 2021, 126 million people worldwide will be affected with inflammatory diseases. Although many treatments for inflammatory

diseases are available, but some new herbal medicine need to be discovered that will have fewer side effects instead of synthetic ones that will cure the disease and also show less side effect [25]. Plant extracts have been used in ethno-medical treatments that have fewer side effects as compared with synthetic treatment.

1.3 Aims and Objectives

The aims of this study is to identify Cyclooxygenase, mPGES-2, HNE and tyrosinase enzyme inhibitors, harmless and natural anti-inflammatory compound from *T. foenum-graecum.* And therefore we focus on protein-ligand interactions, which play a significant role in structural drug design. To achieve the goal we have following objectives:

- To identify various bioactive compounds of *Trigonella foenum-graecum* as potential inhibitors of cyclooxygenases, mPGES-2, HNE and tyrosinase enzymes.

- To analyze the binding conformation between targeted proteins and other inhibitors as standard anti-inflammatory agents.

- To compare the results of inhibitors or ligands with standard anti-inflammatory drugs and selection of lead compound.

Chapter 2

Literature Review

2.1   Inflammation

Inflammation is defense mechanisms that protect tissue from harmful stimuli and it is a complex biological reaction of vascular tissues including possibly harmful external and internal stimuli such as disease causing agents, chemicals and foreign agents. The immune system response to injury and infection process of inflammation is an important part. It is a way of signaling the body's immune system to restore and repair damaged tissues, as well as protecting itself from foreign invaders, such as pathogen (bacteria and viruses) [26]. However, this can be a problem if the inflammation continues for a longer period of time or if the inflammatory reaction occurs in places where it is not needed. Inflammation is a general response, and therefore it is considered as natural immune system, rather than adaptive immunity, specific to each pathogen [27].

2.1.1   Signs And Symptoms of Inflammation

The sign and symptoms can also depend on the condition that contains the inflammatory component. Signs and symptoms of inflammation are often usually characterized by pain, redness, swelling, and loss of function. It can also cause

flu-like symptoms; which are fever, loss of appetite, fatigue, muscle stiffness, and headaches [28].

2.1.2 Types of Inflammation

Inflammations are classified into two main types the one is acute inflammation and the other is chronic inflammation.

2.1.2.1 Acute Inflammation

Acute inflammation is types of inflammation, that is an immediate and early response to injuries and it usually takes only a few days. If a wound gets hot, red, painful and swollen, we think that inflammation is working [29]. Inflammation is a useful process, helping to stimulate the injured area as the rest of the immune system is stimulated to heal. In acute inflammation, innate immune cells form the first defense line of the immune system and regulate the activation of the adaptive immune response.

The important components of acute inflammatory response are leukocytes, cytokines and acute phase proteins (APPs) [30]. Acute inflammation is classified into two stages the one is vascular stage and another is cellular stage. So when the acute inflammation succeeds in eliminating the offender, the reaction is reduced, but if the response fails to eliminate the invaders, it can progress to a chronic stage [31].

2.1.2.2 Chronic Inflammation

Chronic inflammation is also known as a mild, long-term inflammation that takes several months to years. In general, the extent and effects of chronic inflammation vary with the cause of the injury and the body's ability to control damage and repair. Chronic inflammatory diseases are the leading cause of death all over the world. The WHO recognized chronic diseases as the greatest threat to human

health. Worldwide, 3 out of 5 people die from chronic inflammatory diseases [32]. Most features of acute inflammation persist because the inflammation becomes chronic, which include dilation of blood vessels (vasodilation), increased blood flow, capillary permeability and the transfer of neutrophils to the affected tissue through the capillary wall. However, WBC's formation rapidly changes and macrophages, lymphocytes begin to replace short-lived neutrophils [33]. Differences between acute and chronic inflammation was given in Table 2.1.

TABLE 2.1: Given Table Summarized Differences Between Acute And Chronic Inflammation

	Acute Inflammation	**Chronic Inflammation**
Causes	Tissues injuries or harmful pathogen such as bacteria and viruses	Disease causing agents that cannot be broken down by the body, including certain types of viruses, foreign bodies that are present in the nature or promote an immune response.
Onset	Quickly and for short term	Slowly and for long term
Duration	Usually takes a few days	Take several months to years
Outcomes	Inflammation recovers or infection develops or become chronic	Loss of tissue, thickening and damaging of connective tissues.

Symptoms of chronic inflammation are the action of primary inflammatory cells for example macrophages, lymphocytes, and plasma cells into the tissue site, producing inflammatory cytokines, growth factors, enzymes, and therefore causing tissue damage and secondary repair which including fibrosis and granuloma formation etc. Here are some common sign and symptoms of chronic inflammation which are Physical pain, chronic fatigue, insomnia, depression, anxiety and mood disorders, weight gain or weight loss and also gastrointestinal complications such

as constipation and diarrhea are the most common sign and symptoms of chronic inflammation [34]. Chronic inflammation is further classified into two types (i) Specific chronic inflammation (ii) Non-Specific chronic inflammation.

2.1.3 Prevalence And Risk Factor

With significant changes in disease trends and levels in different countries and regions, the burden of inflammatory disease is increasing globally. So estimated the prevalence and number of people affected globally with inflammatory diseases were given in Table 2.2. Understanding these geographical differences is essential to developing effective strategies for the prevention and treatment of inflammatory diseases [35]. In 1990, according to age wise, the prevalence rate increased from (79.5 to 83.3) per 10,000 populations and in 2021 it increased from (79.5 to 89.5). In 2021, there are 126 million cases of inflammatory diseases worldwide reported, so in 2021 the number of cases increased rapidly around globally and specifically 282 cases of inflammatory diseases reported in Pakistan according to WHO [36].

TABLE 2.2: Following Table Estimated the Prevalence And Number of People with Anti-Inflammatory Diseases

WHO Region	Prevalence		Number	
	1990	2021	1990	2021
African Region	2.70%	3.90%	2 million	3 million
Region of the Americas	12.20%	21.50%	1.5 million	2.1 million
Eastern Mediterranean Region	4.00%	12.70%	20 million	32 million
European Region	22·0%	32.10%	9.5 million	12 million
South-East Asia Region	3.40%	5.90%	10.4 million	44 million
Western Pacific Region	11.20%	15.50%	12.3 million	36 million
Total	83.3%	89.90%	102 million	126 million

2.1.4 Treatment of Inflammation

Treatment of inflammatory diseases may include rest, exercise, medication, acupuncture and surgery to repair joint and tissue damages. The treatment plan will depend on a number of factors, including the type of disease, the patient's age and the medication the patient is taking, the patient's overall health and how the symptoms are severe [37]. The treatments of inflammatory diseases are described below.

2.1.4.1 Anti-inflammatory Drugs

There are several anti-inflammatory drugs or medicines which can reduce pain, swelling, prevent blood clot and inflammation. Anti-inflammatory agents, drugs and their mechanism of action were given in Table 2.3. These drugs can prevent or even slow down the inflammation. Anti-inflammatory drugs include; nonsteroidal anti-inflammatory drugs (NSAID's) such as naproxen, aspirin, ibuprofen, acetaminophen (Paracetamol) and Celebrex etc. Some of anti-inflammatory drugs are used to treat inflammatory bowel disease and cancer [38]. Non-steroidal anti-inflammatory drugs (NSAIDs) are mostly prescribed pain medications. NSAIDs are a very effective medicine for pain and inflammation [39].

Additionally anti-inflammatory drug resulting, which having pain-relieving and antipyretic activities. Nonsteroidal anti-inflammatory drugs (NSAIDs) produce their therapeutic activity through inhibition of cyclooxygenase (COX) and Inhibition of other novel enzymes which involve in inflammation [40]. Associated protein cyclooxygenase (COX-2) could also be responsible for the high levels of prostaglandins (PGs) in abundant inflammatory condition. Cyclooxygenase-2 excessive expression metabolizes accumulation of PGE2. Cyclooxygenase-2 is a selective inhibitor that can be a category of non-steroidal anti-inflammatory drugs (NSAIDs) which directly targeted cyclooxygenase-2. So these enzymes are involved in the synthesis of significant biological mediators, which are involved in anti-inflammatory activity [41].

TABLE 2.3: Following Table Shows Anti-Inflammatory Agents and Drugs and Their Action

Agent	Drug	Action
Acetylsalicylic acid	Aspirin (Acetosal)	Aspirin has a number of different several effects on body, which mostly reducing inflammation, pain and reducing fever.
Isobutylpheny lpropionic acid	Ibuprofen (2-hydroxyibuprofen)	Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID). It works by inhibiting body's production of certain natural substances that cause inflammation. This effect helps to reduce swelling, fever and pain.
Acetaminophen	Paracetamol (Tylenol)	Paracetamol (acetaminophen) is typically considered a weak inhibitor to the synthesis of prostaglandins (PG). However, the effects of paracetamol are similar to those of selective (COX-2) inhibitors, paracetamol also reduces the number of PG and also involve in inflammatory pathways.

Celecoxib	Celebrex (Etoricoxib)	A highly selective reversal inhibitor of cyclooxygenase COX-2, silicoxyb inhibits the conversion of arachidonic acid into prostaglandin precursor. Therefore, it is anti-inflammatory and analgesic activity.
Benzeneacetic acid derivative	Diclofenac (Voltaren)	It is the inflammatory, analgesic and antipyretic activity. Like other NSAIDs, the mechanism of action of is not fully recognized but involve in the inhibition of COX-1 and COX-2 enzymes.
Propionic-acid derivative	Naproxen (Aflaxen)	Naproxen works by blocking COX-1 and COX-2 enzymes as inactive Coxib. This results in the inhibition of prostaglandin synthesis. Prostaglandins induce inflammation, acting as signaling molecules.

2.1.4.2 Acupuncture

Acupuncture is a type of treatment that involves inserting very thin needles into specific areas of the body, often staying away from the area of pain. Acupuncture can stimulate the brain to produce endorphins. Endorphins, formed naturally in the brain and they prevent pain sensations and reduce inflammation [42].

2.1.4.3   Surgery

Surgery is another treatment for inflammatory diseases. If joints and tissues are severely damaged by inflammation so it is necessary to remove infected or damage tissue or part through surgery. Most common procedure for surgery are; arthroscopy, osteotomy, replacement of joints and synovectomy etc [43].

2.2   Medicinal Plants

Nature is always a golden representation to show the salient features of coexistence. Plants and animals containing natural products which are specifically used for the treatment of various human diseases [44]. Medicinal plants are currently in demand and their acceptance is slowly increasing. Medicinal plants are those plants which having healing properties and having useful medicinal effects on the human or animal body and these plants also called medicinal herbs [45]. The word medicinal plant refers to a variety of different plants which having Medicinal properties. These plants are a great source of Compounds that can be used to synthesize drugs [46]. Different parts of medicinal plants are used for the development of drugs such as leaf, seeds, root, flower or even whole plant. Mostly medicinal plants contain bioactive compounds which have directly or indirectly therapeutic effects and are used as medicinal agents. So these plants are globally used as complementary or alternative medicine [47].

Generally, medicinal plants have been used since earliest times. It can be said that before the history of medicinal plants, the ancient people used these plants for many purposes such as fuel, clothing, shelter and food [48]. Given the fact that, at that time, it was not enough Information on the causes of the disease, how to use plants, how to use these plants for treatment of various diseases and how to use them for medicinal purposes [49]. But now a day's people generally use medicinal plants and spices because these plants contain a lot of bio-active compounds and essential oils which is beneficial for human health. So these plants and spices

contain special compounds which prevent various diseases. Plant components also having characteristics and ability to prevent the development of certain diseases [50]. Toxic and negative effects of conventional and allopathic medicines have also been a significant factor sudden increase its demand in population as well as the development of herbal medicines decreases the use of chemical drugs [51]. On the other hand, the distribution of medicinal plants worldwide is not same and medicinal herbs are mostly collected from wildlife populations [52]. Therefore, in recent decades, demand for wildlife resources has increased from 8 to15% per year in Europe, North America and Asia [53]. Medicinal plants have a promising future as long as they exist. Nearly half a million plants worldwide, most of them not yet studied in medical practice, So current and future studies on medicinal plants maybe effective for the treatment of diseases [54].

2.3   *Trigonella foenum graceum*

Trigonella foenum graceum (fenugreek) is an annual plant within the family Fabace. Fenugreek is an aromatic herbaceous plant and is a very significant spice and is commonly used as a traditional food and medicine [55]. Fenugreek is one of the world's oldest cultivated spice crops. It has grown for its medicinal purposes. The dried seeds of these plants are extremely used in food and beverages as a flavor and supplement. It is grown all over the world as a dry crop. Its seeds and leaves are common ingredients in transportation from the Indian subcontinent and are mostly used in traditional medicine [56]. The *Trigonella foenum-graecum* edible part of the leaves contains moisture (86.1%), carbohydrates (6%), protein (4.4%), minerals (1.5%), fiber (1.1%) and fat (0.9%). Hence these plants are good source of proteins, fats, carbohydrates, minerals and vitamins for example vitamin A, B1 and C [57].

The seed and leaves of this plant mostly are used for the treatment of many other diseases in several traditional systems together with Ayurvedic medicine. In Ayurvedic system, individually fenugreek seeds and leaves are used to prepare

powder or extract for medicinal purposes. *Trigonella foenum-graecum* cold water extract, is usually used against respiratory infections (pneumonia & bronchitis) and subsequently it nourishes the body during illness, this herb is specifically used to reduce temperature, pain and inflammation [58]. This plant has been shown to possess, hypolipidemic and hypoglycaemic activities. The plant's fenugreek is known for its ability to maintain cholesterol levels, as well as its ability to maintain glucose levels. *Trigonella foenum-graecum* pharmacological properties as well as antiviral, antimicrobial, antioxidant, anti-inflammatory activity have been reported [59]. The pharmacological and biological actions of fenugreek are recognized due to the diversity of its constituents as well as secondary metabolites which is derived additionally called phytochemicals. *T. foenum-graecum* plant and seeds were given in Figure 2.1.

Most important phytochemicals present in fenugreek seeds are carbohydrates, proteins, lipids, alkaloids, flavonoids, fiber and steroidal saponins, etc., which can be classified as volatile and non-volatile ingredients [60]. Hydroxyisoleucine, alkaloid, flavonoid and phytic acid are the derivatives of *T. foenum-graecum*. The alkaloid and flavonoid content of fenugreek seeds might be responsible for the inflammatory effects [61]. Hydroxyisoleucine could be a unique organic compound isolated from fenugreek seeds. Phytic acid is abundant in nature (especially in plants). It accounts for 1to 5% weight of edible cereals, legumes, nuts, oil seeds and these are also derived from fenugreek [62].

2.3.1 Classification of *T. foenum-graecum*

Fenugreek belongs to the Fabaceae (Leguminosae) family, subfamily Papilionaceae and genus *Trigonella*, is a versatile and commercially significant spice crop grown for its seeds, shoots and fresh leaves. According to Hutchinson, the genus *Trigonella*, along with five other genera such as Factorovekya, Medicago, Parachetus, Melilotus and Trifolium of the family Fabaceae. Hundred species of *Trigonella* have been reported [63]. Taxonomic hierarchy of *Trigonella foenum* was given in Table 2.4.

FIGURE 2.1: Given Figure Represents *T. foenum-graecum* Plant And Seeds.

These are the native plant of the Indian subcontinent and the Eastern Mediterranean region. *T. foenum-graecum* edible parts of the leaves contain proteins, fats, carbohydrate, vitamins and minerals. Cultivation of this crop is limited to those areas with moderate or low rainfall and cold growing season without extreme temperature [64]. Fenugreek crop mostly grown in Pakistan, India, Turkey, Egypt, United Kingdom, Canada and the United States [65].

TABLE 2.4: Following Table Shows Taxonomic Hierarchy of *Trigonella foenum graceum*

Serial No	Domains	Eukarya
1	Kingdom	Plantae
2	Sub Kingdom	Viridiplantae
3	Super Division	Embryophyta
4	Division	Tracheophyta
5	Sub Division	Spermatophytina
6	Class	Magnoliopsida
7	Super Order	Rosanae
8	Order	Fabales
9	Family	Fabaceae
10	Genus	*Trigonella L*
11	Species	*Trigonella foenum graceum L*

2.3.2   Medicinal Uses of *Trigonella foenum graceum*

There are several medicinal uses of *T. foenum-graceum*, which play a significant role to cure inflammatory diseases and various other diseases. *T. foenum-graceum* seeds are used in traditional medicine as an antibacterial, anti-inflammatory and antidiabetic. Galactagogue as actually effective against anorexia [66]. The seed and leaves of this plant mostly are used for the treatment of many other diseases in several traditional systems together with Ayurvedic medicine. In Ayurvedic system, individually fenugreek seeds and leaves are used to prepare powder or extract for medicinal purposes [67]. Many of the physical health benefits of fenugreek seeds have been experimentally validated in recent decades in animal studies as well as in human trials. Fenugreek seeds were found to possess anti-inflammatory activities. Multiple pharmacological effects of *Trigonella* plants were given in Figure 2.2. The inflammatory action of fenugreek is due to the inhibition of cyclooxygenase proteins (COX-1 and COX-2) and other novel enzymes [68]. Studies of various infectious diseases and laboratory research have revealed the biological functions of fenugreek. In recent times, medicinal compounds derived from plants have been widely used and have been recommended by doctors for their use in a number of diseases due to their minimal side effects and numerous positive effects on human health. A large number of studies have returned positive results, indicating the effectiveness of fenugreek seed as an active food that can be beneficial to health and disease. Fenugreek is known for its numerous pharmaceutical properties such as anti-inflammatory, antioxidant, antidiabetic, anticancer, antiulcer digestive stimulant, gastro-protection and cardio-protection [69]. The seeds of *Trigonella foenum graceum* were given orally to experimental animals, so it producing a hypoglycemic effect and lowering blood glucose levels. These studies suggest that *Trigonella* can be used as a food supplement to regulate glucose level in the blood [70]. There are various anti-inflammatory agents known to decrease the inflammatory response, such as corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs). Identifying natural, plant-based and non-toxic anti-inflammatory drugs is essential for the treatment of various inflammatory diseases [71].
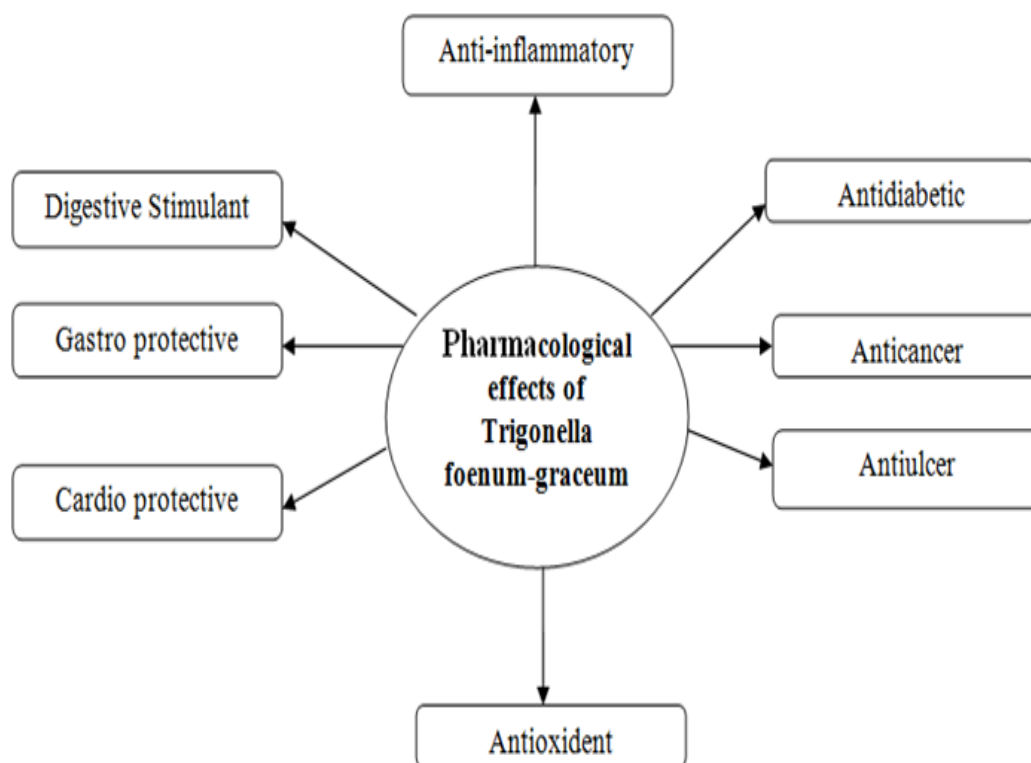
FIGURE 2.2: Given Figure Indicates Multiple Pharmacological Effects of *T.foenum-graceum.*

2.3.3 Bioactive Compounds In *Trigonella foenum graceum* As Inhibitors

Additionally many essential nutrients in plants contain a variety of biologically active non-nutrient groups. "Bioactive compounds" have been described as compounds that cause a specific biological reaction in animal and humans. Bioactive compounds of fenugreek which act as inhibitors are discussed below.

2.3.3.1 Steroid Saponins

Fenugreek plant mostly contains phytoconstituents such as steroid saponins, diosgenin and sapogenins. Steroidal saponins structure consist of 6-C ring with 2 to 3 side chains which having methyl or hydroxyl group. Fenugreek seeds do not contain free sapogenin but are found as a complex glycoside. This is a precursor to the

synthesis of progesterone that was earlier used in combined oral contraceptives. It has the property of lowering serum cholesterol levels [72].

2.3.3.2 Poly Phenol

Poly Phenol is category of chemical compounds which is mainly derived from plants. The result in recent reports concluded that fenugreek seeds consist of five different types of flavonoids which are tricin, vitexin, naringenin and quercetin [73]. These poly-phenolic compounds show strong anti-inflammatory, antioxidant, anticancer and antidiabetic activities and containing many other significant properties. The chemical structure of polyphenols is classified based on the number of phenol rings [74].

2.3.3.3 Alkaloids

Trigonelline is a methyl betaine derivative of necotinic acid which is present in fenugreek seeds, is an important alkaloid. These are a large group of naturally occurring organic compounds which having nitrogen atoms. It is mildly effective and beneficial in the treatment of diabetes and central nervous system disease and also exhibits antibacterial, antiviral, anti-inflammatory and memory-enhancing activities [75].

2.3.3.4 Flavonoids

Flavonoid compounds are plant-derived products and are found in many parts of plants and they are large family of polyphenolic compounds. Inflammatory functions of flavonoids in vitro or in cellular models include synthesis inhibition and stopping the activity of different pro-inflammatory mediators for example; cytokines, adhesion molecules and eicosanoids. The significant role of flavonoids is to inhibit the key enzymes and transcription factors which are involve in inflammation [76].

2.3.3.5   4-Hydroxyisoleucin

4-Hydroxyisoleucin is most widely found free amino acid in fenugreek seeds. It shows analgesic activities and also having a significant role in glucose uptake. It is found in two isomeric forms, the formation of large isomer (2S, 3R and 4S) which gives 90% of in the fenugreek seeds. While minor isomers have [2R, 3R, 4S] formations, that having hypoglycemia and analgesic properties [77].

2.3.3.6   Phytic Acid

Phytic acid is abundant in nature (especially in plants). It accounts for 1to 5% weight of edible cereals, legumes, nuts, oil seeds and these are also derived from fenugreek seeds. Phytic acid (PA) is a naturally occurring ingredient that has been shown to protect against Parkinson's disease (PD). Inflammation in the central nervous system (CNS) is strongly associated with neuronal death in Parkinson's disease. So these all are the bioactive compounds which are present in *Trigonella foenum greacum* are used as inhibitors [78].

2.3.3.7   Quercetin

Quercetin is a plant flavonol of the flavonoid group of polyphenols. It is found in many plants and foods. The flavonoid quercetin was effectively isolated from the leaves of *Trigonella foenum greacum* and its anti-inflammatory and antioxidant activity were studied. Therefore, after a physicochemical analysis of fenugreek leaves, quercetin found in the aerial part of *Trigonella foenum-graecum*. Quercetin is the most abundant dietary flavonoid derived from plants. This bioactive compound has strong ability to inhibit those proteins which play a vital role in infections.

So this bioactive compound has antioxidant and anti-inflammatory effects that reduce inflammation, kill cancer cells, control blood sugar and can help to prevent cardiovascular disease.

2.3.3.8   Trigonelline

Trigonelline is an alkaloid with chemical formula C7H7NO2. Trigonelline is a chemical compound derived from fenugreek seed. These chemical compounds prevent inflammation and protects cells in diabetes by inhibiting fetal growth during pregnancy with leptin and insulin.

2.3.3.9   Tricin

Tricin is a chemical compound. It is an O-methylated flavone, a type of flavonoid. Tricin is bioactive metabolites which is isolated from fenugreek seed. Tricin has been shown to interfere with TLR4 activation by inhibiting proteins to stimulate inflammatory cascades.

2.3.3.10   Naringenin

Naringenin is a flavonoid belonging to flavones subclass. They are isolated from fenugreek as well as from plant *Trigonella foenum greacum*. Naringenin can play a protective role by minimizing mucous production during airway inflammation by regulating the production of ROs and inhibiting NF-kB activity.

2.3.3.11   Flavonol

Most important phytochemical present in fenugreek seeds is flavonol. Flavonols are polyphenols belonging to the class of flavonoids. Flavonol is the derivatives of *T. foenum-graecum*. There are several medicinal uses of *T. foenum-graceum*, which play a significant role to cure inflammatory diseases and various other diseases. The flavonol content of fenugreek seeds might be responsible for the inflammatory effects. Flavonol use their anti-inflammatory activities by decreasing the production of reactive oxygen species (ROS) and the down-regulation of numerous inflammatory mediators through inhibition of signaling pathways.

2.4 Targeted Proteins

There are 4 different types of proteins which are used as targeted proteins for molecular docking process such as Cox-2, mPGES-2, HNE and Tyrosinase.

2.4.1 Cyclooxygenases

Cyclooxygenase enzyme also called Prostaglandins (PTGS), which is responsible for the formation of prostanoids, together with arachidonic acid from prostaglandins such as Thromboxane and Prostaglandin. The mechanism when cyclooxygenase form prostanoids, together with arachidonic acid from prostaglandins was given in Figure 2.3. There are 2-isoforms of cyclooxygenase that are known as cyclooxygenase-1 and Cyclooxygenase-2. Cyclooxygenase-1 functions as a housekeeping isoform of Cyclooxygenases and is mainly expressed to serve functions resembling controlling of urinary organ blood flow, stomach protection against ulcers and preparation of prostaglandin E-2 [79].

On the other hand Cyclooxygenase- 2 is associated in an inducible initial response which is activated in response to genes and numerous extracellular or intracellular physiological stimuli. Cyclooxygenase-2 is an important mediator of the inflammatory pathway. Associated protein cyclooxygenase (COX-2) could also be responsible for the high levels of PGs in abundant inflammatory condition. Cyclooxygenase-2 excessive expression metabolizes accumulation of PGE2 [80]. Cyclooxygenase-2 is a selective inhibitor that can be a category of non-steroidal anti-inflammatory drugs (NSAIDs) which directly targeted cyclooxygenase-2. The molecules that targeted the flow of prostaglandin synthesis-2 regulate many signaling pathways and control apoptotic proteins and thus contribute to numerous physiological processes as well as proliferation, survival, mutation and metastasis. And therefore the excessive-expression and up-regulation of cyclooxygenase-2 are mostly connected with inflammation, unchecked cell division and cell death. Fenugreek seeds were found to possess anti-inflammatory activities. The inflammatory action of fenugreek is due to the inhibition of cyclooxygenase proteins (COX-1 and
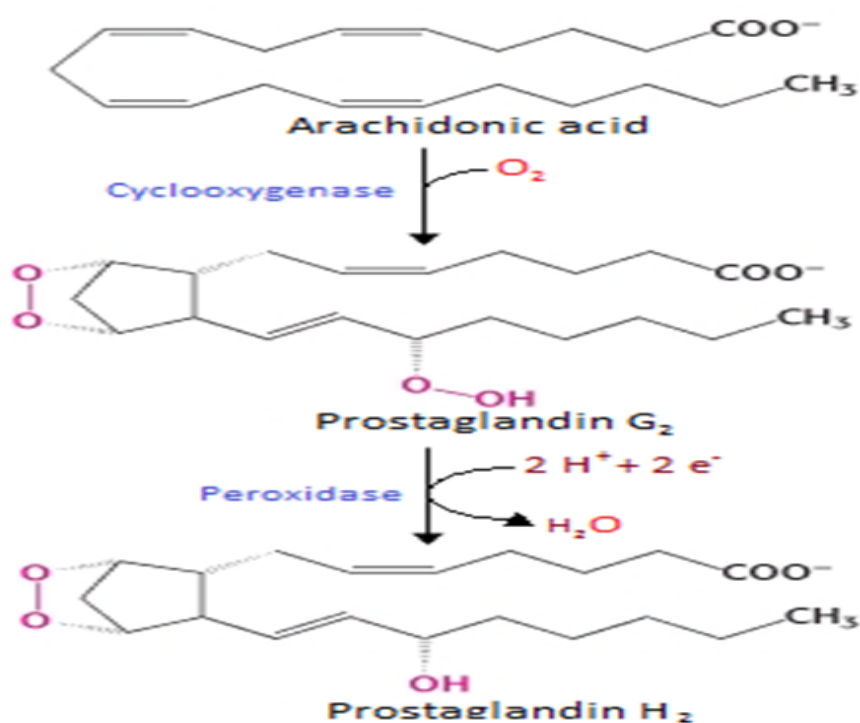
FIGURE 2.3: Mechanism when Clooxygenase form Prostanoids, together with Arachidonic Acid from Prostaglandins

COX-2) and lipid peroxidation due to the inhibition of flavonoids-C-glycosides, triglycerides and fatty acids present inside the seeds [81].

2.4.2 Microsomal PGES-2

Microsomal prostaglandin E synthase-2 (mPGES-2) is an eicosanoid lipid mediator that significantly contributes to the pathogenesis of many inflammatory diseases [82]. PGES-2 is formed when phospholipase secreted arachidonic acid from the plasma membrane and contains two cyclooxygenases such as cyclooxygenase-1 and cyclooxygenase-2 and specific isomers, like mPGES-1 and mPGES-2 [83]. The most commonly used nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, silicoxyb and acetaminophen (Paracetamol) have been shown to block inflammation, pain, and fever by blocking cyclooxygenase activity and thus reducing the generation of PGE-2 in injured tissues and proven useful in the treatment of Nervous system [84]. Consequently, the flow pathway elements of cyclooxygenase

enzymes in the biosynthetic reaction of PGE-2, terminal synthesis and receptors are considered more specific targets for the treatment of pain and inflammation. Out of three-terminal synthesis, mPGES-1 frequently combines functionally with COX-2 and is the primary source of inflammatory prostaglandin E- 2 synthesis. Inhibition of mPGES-1 has shown similar effects with NSAIDs in various experimental animal models. However, such an approach would actually protect the analgesic efficacy expected from NSAIDs, which needs further verification. Prostaglandin E synthase-2 combines with four specific G proteins to act on receptor subtypes, called EP1–4 receptor. Targeting four receptors which are also being followed in the development of analgesics as an alternative approach to CoX-2 inhibitors. Targeting mPGES-1 and possibly four PGE-2 receptors could serve as promising strategies for the evolution of anti-inflammatory drugs for the next generation [86].

2.4.3   Human Neutrophil Elastase (HNE)

Human neutrophil elastase (HNE) is a protease enzyme that belongs to the chemo trypsin like family of serine proteins. These enzymes play a vital part in the pathogenesis of many infections, from chronic inflammatory diseases to infectious diseases [87]. Neutrophils survive quickly in the blood of healthy people (round about 8 hours), but after inflammation they become active and their longevity can increase up to 5 days. When inflammatory stimuli develop in peripheral tissues, neutrophils are quickly recruited to the anatomical location of inflammation. Neutrophil degranulation is responsible for the release of various inflammatory mediators for example neutrophil elastase, catechin G and proteinase-3.

2.4.4   Tyrosinase

For melanin biosynthesis tyrosinase is a key enzyme which plays a significant role in skin inflammation. It was first identified by French chemist Gabriel Bertrand. Several acute or chronic inflammatory skin reactions can produce a change in

skin color [88]. Melanosome are unique organs to be found in the cytoplasm of melanocytes, containing important enzymes that control the pigmentation of skin color, such as tyrosinase enzymes and its type's TYRP-1 and TYRP-2.

2.5 Molecular Docking

Molecular Docking is technique used to estimate the strength of a bond between a ligand and a target protein through a special scoring function and to determine the correct structure of the ligand within the target binding site. The 3D structure of the target proteins and the ligands is taken as the input for docking. It represents a frequently used approach in structure-based drug design since it requires a 3D structure of a target protein. It can be used to determine the correct structure of the ligand within the target binding site, and to estimate the strength of the binding between the ligand and the target proteins through a specific scoring function [89]. It also helps in the recognition of new small molecular compounds, revealing the essential properties, such as high interaction between binding with target protein having reasonable absorption, distribution, metabolism and excretion, which help in the selection of lead for the target [90]. So the docking process includes compounds which are discussed below.

- The docking process requires a 3D structure of protein which is downloaded from protein data bank (PDB).

- Minimum size of molecules or compounds or virtual compounds that contain a database is required.

- A computational framework is also needed to perform the docking and find the scoring process.

Protein and ligand docking is one of the key areas of molecular docking, which is obtain high popularity and appreciation due to its role in structure-based drug designing. Molecular dynamics, distance geometry method and genetics algorithm

etc are most widely used algorithm in molecular docking and the most frequent software used for molecular docking were Auto Dock vina, Auto Dock, CB Dock and ICM etc.

Chapter 3

Methodology
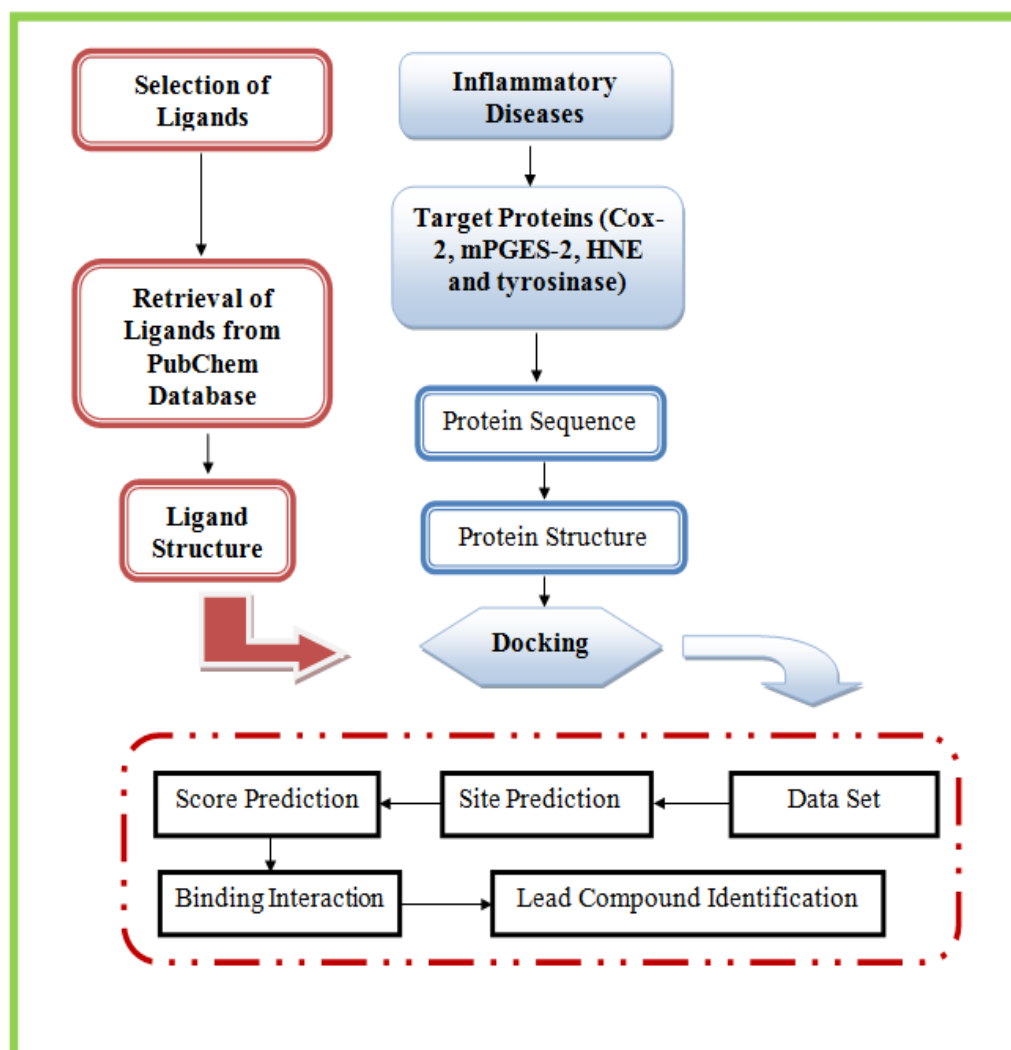
FIGURE 3.1: Flow Chart of Methodology (A).
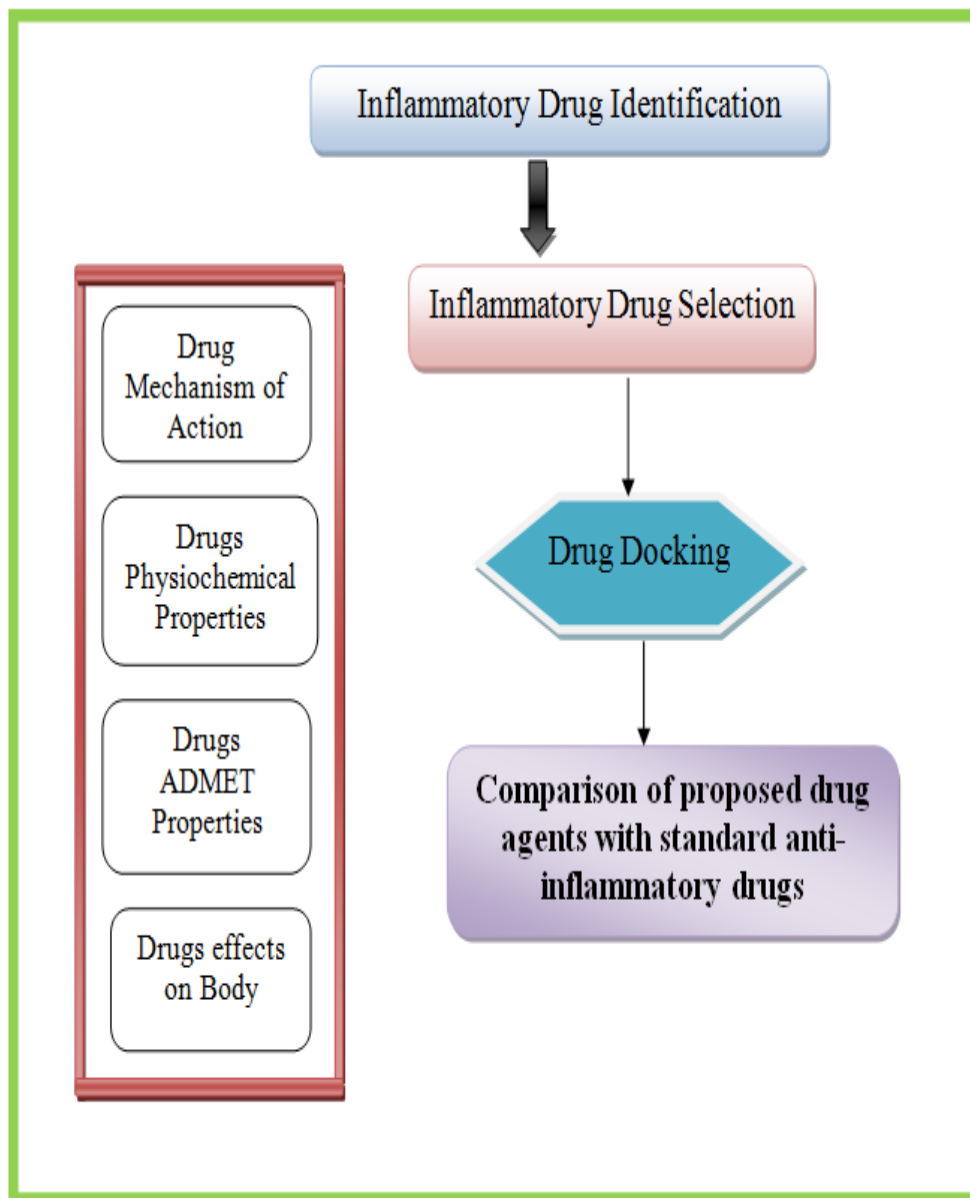
FIGURE 3.2: Flow Chart of Methodology (B).

3.1 Disease Selection

Inflammation is a physiological condition in which a part of the body becomes red, swollen and painful, particularly in response to an injury or infection which causes immune dysfunction, tissue damages etc. Cyclooxygenases and other novel enzymes are involved in inflammatory process, which play a key role in inhibiting inflammatory pathways. A significant progress has been made in understanding the role of these enzymes in various biological processes. Inflammatory diseases

are the most important infectious disease worldwide [91].

3.2   Target Proteins Selection

It is possible to manage inflammatory diseases, the key factor involved, which are enzyme cyclooxygenases and other novel enzymes. So these enzymes are involved in inflammatory pathways which play a vital role to inhibit inflammation. The increasing of inflammation can cause certain diseases such as cancer, inflammatory bowel disease and chronic peptic ulcer etc [92]. Selection of primary sequence (FASTA format) of target proteins (Cox-2, mPGES-2, HNE and tyrosinase) from UniProt database were taken with accession number Q05769, Q9N0A4, P08246, P14679 and 604, 377, 267, 529 residues length, which showed that 3D structure of Cyclooxygenas-2, mPGES-2, HNE and tyrosinase is available in PDB (Protein Data Bank) [93].

3.3   Primary Sequence Retrieval

Primary sequence of target proteins (Cox-2, mPGES-2, HNE and tyrosinase) Were taken in FASTA format from protein sequence database UniProt under accession number P35354, Q9H7Z7, P08246, P14679 and 604, 377, 267, 529 residues length [94].

3.4   Analysis of Physiochemical Properties

Physicochemical properties play a significant role in determining the function of proteins. ProtParam was used to predict these properties of Cyclooxygenas-2, mPGES-2, HNE and tyrosinase enzymes [95]. The number of positively charged residue (Arg+ Lys) and negative charged residue (Asp+ Glu), theoretical pI, molecular weight, Ext.coefficient (Cys included), Ext.coefficient (Cys not included),

instability index, aliphatic index and grand average of hydropath-city were computed through ProtParam [96].

3.5 Functional Domain Identification of Targeted Proteins

Interpro is an online database which was used to identify the functional domains of Cox-2, HNE tyrosinase and mPGES-2 [97]. Conserved domains are involved in sequence/structure/relationship.

3.6 3D Structure Predictions of Protein

I-TASSER Iterative Threading ASSEmbly Refinement is an online protein 3D structure and function prediction server. This online server firstly identifies the structural model of the PDB through multiple threading approaches, including full-length atomic models that were constructed using Iterative threading fragment assembly simulations.

This server predicts regions of secondary structure from the protein sequence such as alpha helix, beta sheet and coil from the amino acid sequence [98]. The I-TASSER server also predict the 3D structure of proteins and these server gives us five 3D structure of proteins so on the basis of c-score we can select the best 3D structure of the protein.

3.7 Retrieval of Chemical Structure of Ligands

PubChem is the world's largest repository of easily accessible chemical information database. So the chemical compounds that were used as ligand were selected from

PubChem database [99]. The selected ligands were refined through Chem Draw Ultra version 12.0.2 software.

3.8 Energy Minimization of Ligands

Energy minimization of ligands were carried out by chem pro software (chem 3D v 12.0.2). This are a mandatory step in the preparation of ligands for docking because unstable ligands will show unreliable vina scores in docking results.

3.9 Bioactivity Analysis of Ligands and Toxicity Measurement

Chemical compounds that are used as ligand were selected from PubChem database. Selected compounds follow the Lipinski rule of five and those are likely to be used as active drug in humans [100]. The potential success of a compound depends on its ADMET properties. PkCSM is an online tool that helps to find the ADMET properties of the compounds. The rules are as follow:

- The logP value of most "drug-like" molecules should be limited to 5.

- Molecular weight should be under 500.

- Maximum number of H-bond acceptor should be 10.

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

3.10 Molecular Docking of Targeted Proteins

The purpose of molecular docking is to find the best conformational interaction between target proteins and compounds. The two essential requirements for docking are the target protein and the candidate ligand. Cyclooxygenas-2, mPGES-2,

HNE and tyrosinase is used as the target protein and selected ligands are alkaloid, flavonoid, phytic acid, 4-hydroxyisoleucin, sapogenin, quercetin, trigonelline, tricin, naringenin and flavonol. CB dock is an online docking server which automatically identifies binding sites and is used to perform docking. It can simplify docking procedures and improve accuracy by predicting target protein binding sites [101].

3.10.1 Process of Molecular Docking

The first step in performing the docking process is to create a ligand and target protein files. First, the target protein file is compiled following a few steps. PDB file of target proteins (Cox-2, mPGES-2, HNE and tyrosinase) were given to CB dock as input file. After these amendments target protein file was saved in pdbqt format. Compilation of protein file the ligands file has been prepared by following the same procedure and saved in pdbqt format in same directory. Then Setting up Grid box around protein ligand structure is performed [102]. Docking files were created for chosen data set after completion of this step and results were saved in pdbqt format.

3.10.2 Active Site Identification

The ligand shows maximum or highest interaction with the protein where the target protein has their active site. Amino acids are highly involved in the formation of complex of ligand to protein. Protein binding pockets were identified by CASTp [103].

3.11 Protein Ligand Interaction

The interaction of the active pockets of the ligand and the protein are calculated for the interpretation of docking results. Two types of interactions are studied;

hydrogen bonding and hydrophobic bonding. Using Ligplot plus (version v.1.4.5) the protein ligand interaction were studied. This software automatically generates schematic diagrams of the protein-ligand interaction of the given ligands in the PDB file [104].

3.12   Ligand ADME Properties

Generally for more successful drug discovery lead needs to be more like drug. The compounds were further screened on the basis of drug score, drug-likeness and toxicity. PkCSM was used for ADMET studies, these server having ADMET properties of a drug [105].

3.13   Lead Compound Identification

After a detailed analysis of protein and ligand interactions, docking scores and toxicity studies, the most active inhibitor was identified. The selected compound was our lead compound.

3.14   Inflammatory Drug Identification

The inflammatory drug identification refers to the identification of drugs that were used for inflammatory diseases, treatment purpose. KEGG and Drug Bank databases were used for drug identification because it helps to analyze the disease in details with its pathway and drugs [106].

3.15   Inflammatory Drug Selection

The identified drugs must be filtered in order to select the most effective drug.

This is done through a detailed study of identified drugs and most effective drug is identified setting parameters; physiochemical properties, effective ADMET properties, effective mechanism of action and minimal side effects. Physical Chemical Properties, ADMET Properties and mechanisms of action with drug side effects were collected from PubChem, Drug Bank, PKCSM, and KEGG databases, respectively [107].

3.16   Anti-Inflammatory Drug Docking

The identified drug then docked with Cox-2, mPGES-2, HNE and tyrosinase protein to identify the inhibition efficiency. CB dock (Cavity-detection guided Blind Docking) is an online docking server which was used to perform docking. It can simplify docking procedures and improve accuracy by predicting target protein binding sites [108].

3.17   NSAID Drug-Proposed Anti-inflammatory Agent Comparison

The comparison between non-steroidal anti-inflammatory drug and the proposed anti-inflammatory agents was done through comparing docking values, physiochemical properties and ADMET properties [109].

Chapter 4

Results and Discussions

4.1 Structure Modeling

4.1.1 Primary Sequence Retrieval

Primary sequence of target proteins (Cox-2, mPGES-2, HNE and tyrosinase) were taken in FASTA format from UniProt database (http://www.uniprot.org) under accession number P35354, Q9H7Z7, P08246, P14679 and 604, 377, 267, 529 residues length.

>sp—P35354—PGH2- HUMAN Prostaglandin G/H synthase 2 OS=Homo sapiens OX=9606 GN=PTGS2 PE=1 SV=2

MLARALLLCAVLALSHTANPCCSHPCQNRGVCMSVGFDQYKCDCTRTGFY
GENCSTPEFLTRIKLFLKPTPNTVHYILTHFKGFWNVVNNIPFLRNAIMSYL
TSRSHLIDSPPTYNADYGYKSWEAFSNLSYYTRALPPVPDDCPTPLGVKGK
KQLPDSNEIVEKLLLRRKFIPDPQGSNMMFAFFAQHFTHQFFKTDHKRGPA
FTNGLGHGVDLNHIYGETLARQRKLRLFKDGKMKYQIIDGEMYPPTVKDT
QAEMIYPPQVPEHLRFAVGQEVFGLVPGLMMYATIWLREHNRVCDVLKQE

HPEWGDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNKQ
FQYQNRIAAEFNTLYHWHPLLPDTFQIHDQKYNYQQFIYNNSILLEHGIT
QFVESFTRQIAGRVAGGRNVPPAVQKVSQASIDQSRQMKYQSFNEYRKR
FMLKPYESFEELTGEKEMSAELEALYGDIDAVELYPALLVEKPRPDAIFG
ETMVEVGAPFSLKGLMGNVICSPAYWKPSTFGGEVGFQIINTASIQSLIC
NNVKGCPFTSFSVPDPELIKTVTINASSSRSGLDDINPTVLLKERSTEL.

>sp—Q9H7Z7—PGES2- HUMAN Prostaglandin E synthase 2 OS=Homo sapiens
OX=9606 GN=PTGES2 PE=1 SV=1

MDPAARVVRALWPGGCALAWRLGGRPQPLLPTQSRAGFAGAAGGPSPVA
AARKGSPRLLGAAALALGGALGLYHTARWHLRAQDLHAERSAAQLSLSSR
LQLTLYQYKTCPFCSKVRAFLDFHALPYQVVEVNPVRRAEIKFSSYRKVPI
LVAQEGESSQQLNDSSVIISALKTYLVSGQPLEEIITYYPAMKAVNEQGKEVT
EFGNKYWLMLNEKEAQQVYGGKEARTEEMKWRQWADDWLVHLISPNVY
RTPTEALASFDYIVREGKFGAVEGAVAKYMGAAAMYLISKRLKSRHRLQDN
VREDLYEAADKWVAAVGKDRPFMGGQKPNLADLAVYGVLRVMEGLDAFD
DLMQHTHIQ.

>sp—P08246—ELNE- HUMAN Neutrophil elastase OS=Homo sapiens OX=9606
GN=ELANE PE=1 SV=1

MTLGRRLACLFLACVLPALLLGGTALASEIVGGRRARPHAWPFMVSLQLRGG
HFCGATLIAPNFVMSAAHCVANVNVRAVRVVLGAHNLSRREPTRQVFAVQRI
FENGYDPVNLLNDIVILQLNGSATINANVQVAQLPAQGRRLGNGVQCLAMGW
GLLGRNRGIASVLQELNVTVVTSLCRRSNVCTLVRGRQAGVCFGDSGSPLVC
NGLIHGIASFVRGGCASGLYPDAFAPVAQFVNWIDSIIQRSEDNPCPHPRDPD
PASRTH.

>sp—P14679—TYRO- HUMAN Tyrosinase OS=Homo sapiens OX=9606 GN=TYR PE=1 SV=3

MLLAVLYCLLWSFQTSAGHFPRACVSSKNLMEKECCPPWSGDRSPCGQLSGR
GSCQNILLSNAPLGPQFPFTGVDDRESWPSVFYNRTCQCSGNFMGFNCGNCK
FGFWGPNCTERRLLVRRNIFDLSAPEKDKFFAYLTLAKHTISSDYVIPIGTYGQ
MKNGSTPMFNDINIYDLFVWMHYYVSMDALLGGSEIWRDIDFAHEAPAFLPW
HRLFLLRWEQEIQKLTGDENFTIPYWDWRDAEKCDICTDEYMGGQHPTNPNL
LSPASFFSSWQIVCSRLEEYNSHQSLCNGTPEGPLRRNPGNHDKSRTPRLPSSAD
VEFCLSLTQYESGSMDKAANFSFRNTLEGFASPLTGIADASQSSMHNALHIYMN
GTMSQVQGSANDPIFLLHHAFVDSIFEQWLRRHRPLQEVYPEANAPIGHNRES
YMVPFIPLYRNGDFFISSKDLGYDYSYLQDSDPDSFQDYIKSYLEQASRIWSWL
LGAAMVGAVLTALLAGLVSLLCRHKRKQLPEEKQPLLMEKEDYHSLQSHL.

Cyclooxygenas-2, mPGES-2, HNE and tyrosinase were selected as the target proteins and alkaloid, flavonoid, phytic acid, 4-hydroxyisoleucin, sapogenin, quercetin, trigonelline, tricin, naringenin and flavonol were selected as a ligands for the current study.

4.1.2 Physiochemical Characterization of Cyclooxygenas-2, mPGES-2, HNE and Tyrosinase

ProtParam is an online tool which allows the calculation of different physical and chemical parameters for a given protein stored in Swiss-Prot or TrEMBL or for protein sequence entered by the user. The various parameters computed by ProtParam are molecular weight, theoretical PI, amino acid composition (positive and negative charge), atomic compound, extinction coefficient, estimated half-life, instability index, aliphatic index, and grand average of hydropathicity (GRAVY). The Calculated pI greater than 7 represents the basic nature of the protein while less than7 represents the acidity of the protein. Light absorption is represented by extinction coefficient.

Instability index which is less than 40 indicates the stability of the protein while greater than 40 indicates the instability of protein [110]. The aliphatic index represents the aliphatic content of a protein. The high value of the aliphatic index indicates the thermo stability of the protein. Molecular weight contains both positive and negative charged residues of protein. At 280nm the ranging extinction coefficient of 73980, 67965, 20105 and 112270 indicates Tyr and Trp high concentration [111]. Low GRAVY shows better interaction with water molecules. Lowest gravity shows better interaction with water molecules. The Physiochemical properties of Cyclooxygenase-2, Microsomal Prostaglandin E synthase 2, Human Neutrophil Elastase and Tyrosinase were shown in Table 4.1, 4.2, 4.3 and 4.4 respectively. So all these parameter which were selected for present study were taken according to earlier research work [112].

TABLE 4.1: Physiochemical Properties of Cyclooxygenase-2 (Cox-2).

MW	PI	NR	PR	
68996.12	7.02	62	61	
Ext.Co1	**Ext.Co2**	**Instability index**	**Aliphatic index**	**GRAVY**
73980	73230	37.67	80.70	-0.287

TABLE 4.2: Physiochemical Properties of Microsomal Prostaglandin E Synthase-2 (mPGES-2).

MW	PI	NR	PR	
39964.88	9.23	36	44	
Ext.Co1	**Ext.Co2**	**Instability index**	**Aliphatic index**	**GRAVY**
67965	67840	44.87	87.08	-0.236

TABLE 4.3: Physiochemical Properties of Human Neutrophil Elastase (HNE).

MW	PI	NR	PR
28518.06	9.71	13	24

Ext.Co1	Ext.Co2	Instability index	Aliphatic index	GRAVY
20105	19480	47.88	102.25	0.237

TABLE 4.4: Physiochemical Properties of Tyrosinase.

MW	PI	NR	PR	
60393.27	5.71	57	44	

Ext.Co1	Ext.Co2	Instability index	Aliphatic index	GRAVY
112270	111270	56.76	71.76	-0.356

MW indicate (Molecular weight), pI indicate (Theoretical pI), NR indicate total number of negatively charged residues (Asp + Glu), PR indicate total number of positively charged residues (Arg + Lys), ExtCo1 indicates (Ext. coefficient) all Cys residues are reduced, ExtCo2 indicates (Ext. coefficient) all Cys residues are reduced, II indicate (instability index), AI indicate (Aliphatic index) and GRAVY indicate (Grand average of hydropathicity).

4.1.3   3D Structure Predictions of Protein

I-TASSER Iterative Threading ASSEmbly Refinement is an online protein 3D structure and function prediction server. This online server firstly identifies the structural model of the PDB through multiple threading approaches, including full-length atomic models that are constructed using Iterative threading fragment assembly simulations.

Although the server has been widely used for various biological and biomedical investigations, this server predicts regions of secondary structure from the protein sequence such as alpha helix, beta sheet and coil from the amino acid sequence [113]. These online server also predict the 3D structure of proteins and gives us

five 3D structure of proteins, so on the basis of c-score we can select the best 3D structure of the protein.

4.1.4   Functional Domain Identification of Proteins

The functional domain is the active part of a protein that is involved in the interaction of proteins with other substances. Proteins can contain more than one active domain that performs different functions. Functional domains of proteins with residues length were shown in Figure 4.1, 4.2, 4.3 and 4.4 respectively.

Cyclooxygenase contain two functional domains by the name of Epidermal Growth Factor like domain and An-peroxidase domain belong to Cox-2 family first one starting from residue number 21 and ends at 53 and another one starting from residue 201and ends at 560.

Cyclooxygenase acts as a homodimer in which each subunits of the dimer consists of epidermal growth factor domain and catalytic domain. In the catalytic domain, there are active sites of COX and peroxides on both sides of the heme synthetic group [114]. Other enzymes such as mPGES-2, HNE and tyrosinase also contain functional domains from which active site of proteins are easily identified. Microsomal Prostaglandin E synthase 2 contain one functional domain by name of Glutathione S- transferase, N-terminal domain belong to PGES-2 family starting from residue number 104 and ends at 172 [115].

Human Neutrophil Elastase contain one functional domain by name of Trypsin domain belong to Peptidase-S1A family starting from residue number 30 and ends at 242. Tyrosinase contain one functional domain by name of Central domain of tyrosinase belong to Copper protein family starting from residue number 170 and ends at 403. Functional domain identification of Cox-2, m PGES-2, HNE and Tyrosinase were represented in Table 4.5.
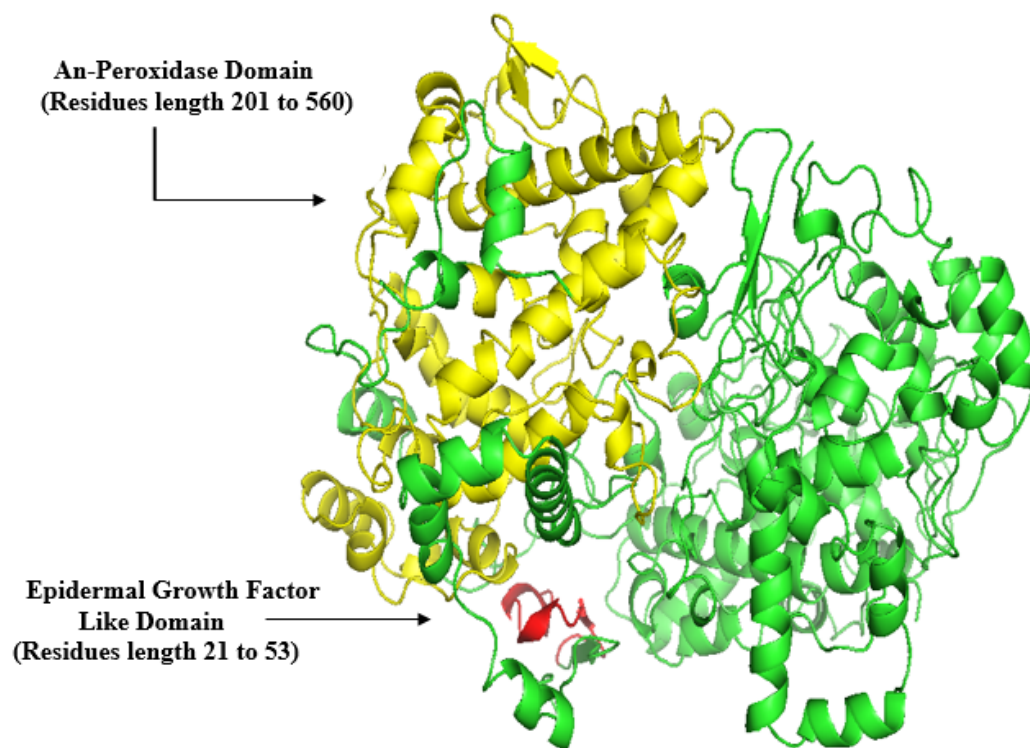
FIGURE 4.1: Two Functional Domains of Cox-2 with Residues Length
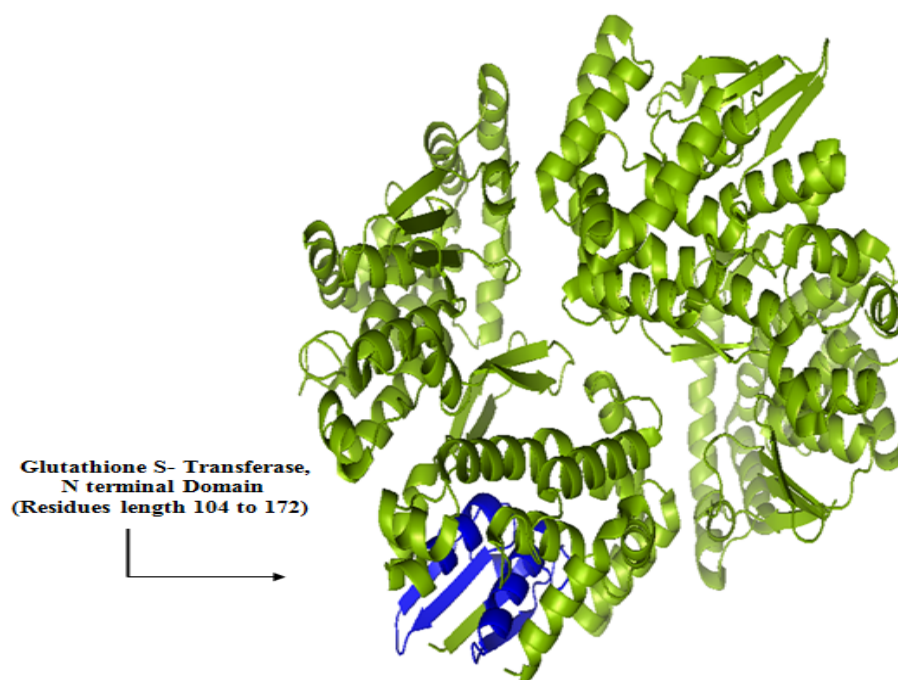
FIGURE 4.2: One Functional Domain of Mpges-2 with Residues Length
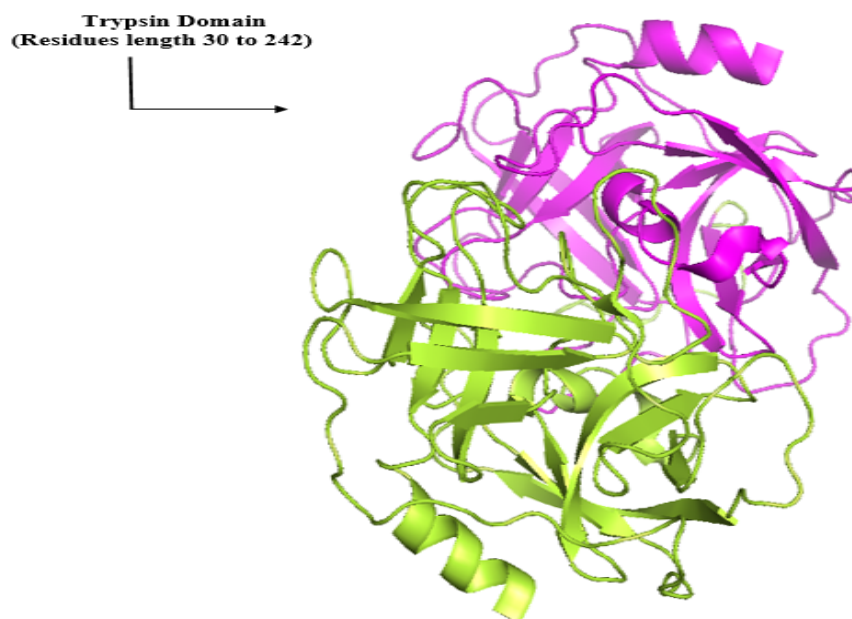
FIGURE 4.3: One Functional Domain of HNE with Residues Length.
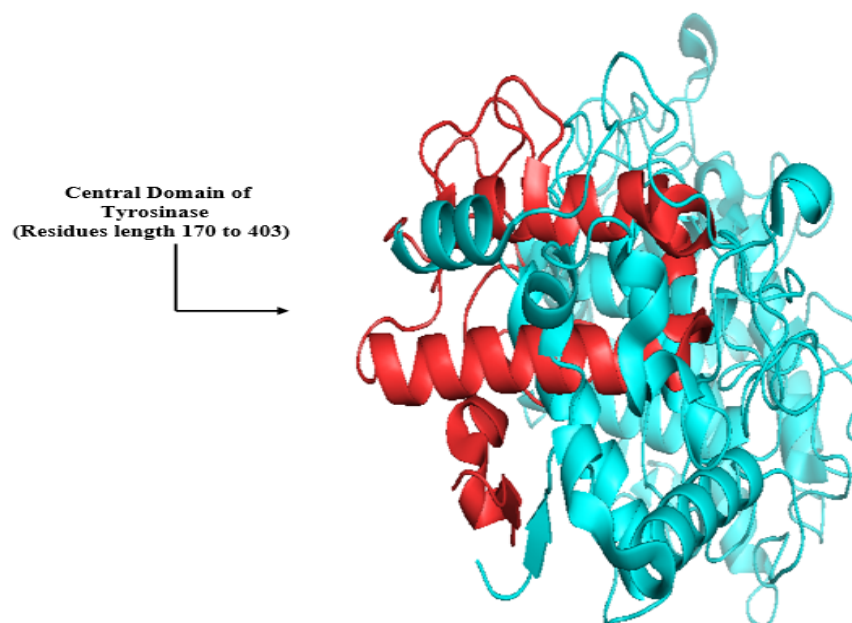
FIGURE 4.4: One Functional Domain of Tyrosinase with Residues Length.

TABLE 4.5: Functional Domain Identification of Cox-2, m PGES-2, HNE and Tyrosinase

S.No	Name	Domain	Start	End
1	Cyclooxygenase-2	Epidermal Growth Factor & An-peroxidase	21&201	53 & 560
2	Microsomal Prostaglandin E synthase 2	Glutathione S- transferase, N-terminal	104	172
3	Human Neutrophil Elastase	Trypsin	30	242
4	Tyrosinase	Central domain of tyrosinase	170	403
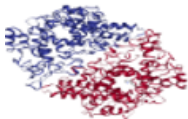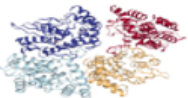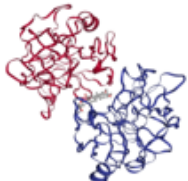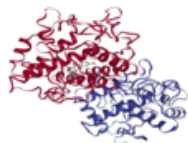
4.1.5   Template Selection

Once a list of possible templates is obtained using search methods, it is important to select one or more templates that are particularly suitable for molecular docking. There are several factors to consider when choosing a template. The simplest template selection rule is to choose the structure that matches to the modeled sequence. If possible, a template bound to the same or similar ligands as a model should mostly be used. The structures of the selected templates are taken from the Protein Data Bank (PDB) and were listed in the Table 4.6.

4.1.6   Structure of Proteins Refined for Docking

The final structure of Cox-2, m PGES-2, HNE and Tyrosinase is refined which is used for docking purposes. All the refined 3D structure of proteins was shown in Figure 4.5, 4.6, 4.7 and 4.8 respectively.

TABLE 4.6: Selected PDB Templates Structures

S.No	Templates	Resolution	PDB ID	Structure
1	Cyclooxygenase-2 (Prostaglandin Synthase-2) complexed with selective inhibitors, SC-558 In I222 space group.	2.80 Å	6COX	
2	Microsomal prostaglandin E synthase type-2.	2.60 Å	1Z9H	
3	Crystal structure of highly glycosylated human leukocyte elastase in complex with a thiazolidinedione inhibitor.	2.70 Å	6F5M	
4	Crystal structures of copper-depleted and copper-bound pro-tyrosinase.	2.05Å	3W6Q	
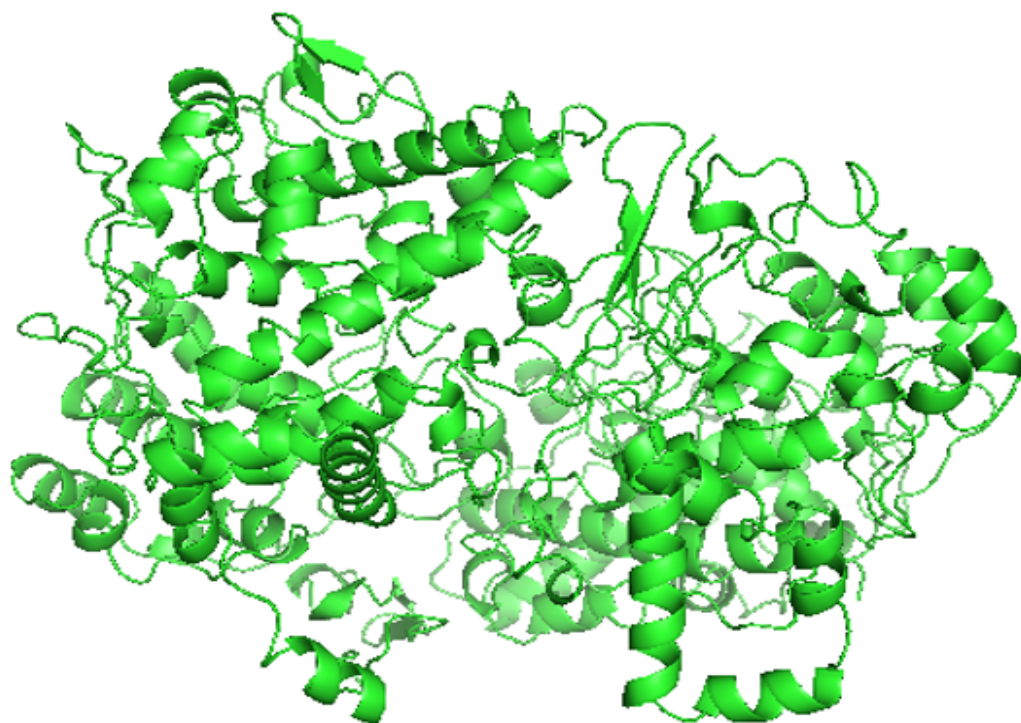
FIGURE 4.5: Given Figure Represented 3d Structures of Cox-2
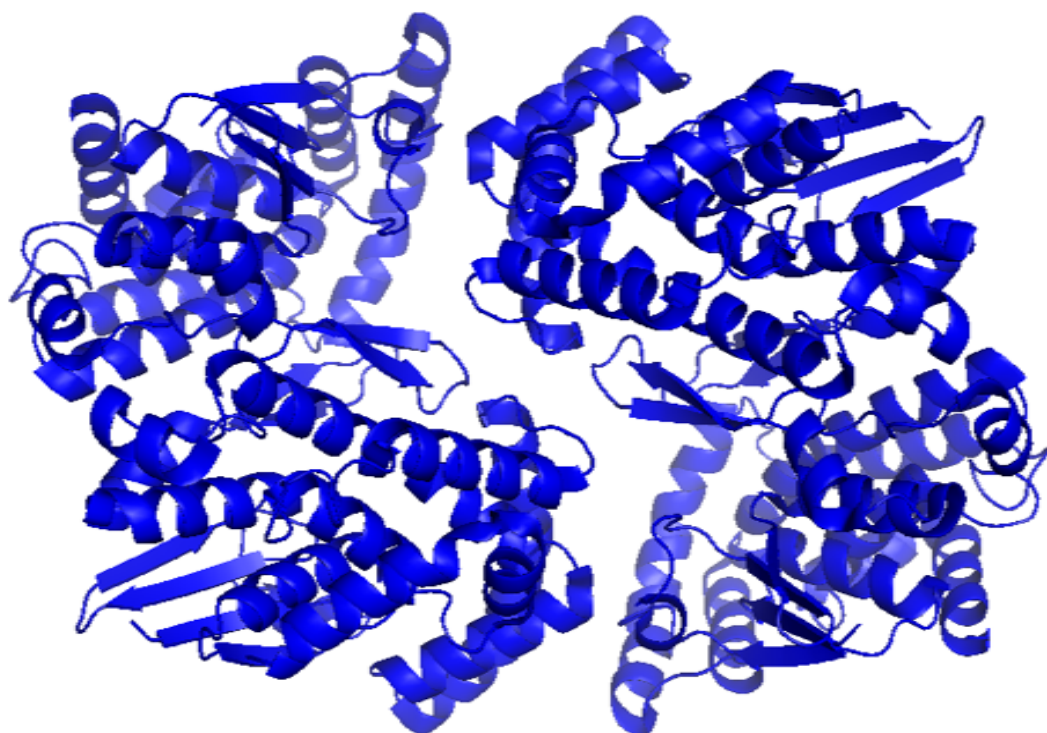
FIGURE 4.6: Given Figure Represented 3d Structures of mPGES-2
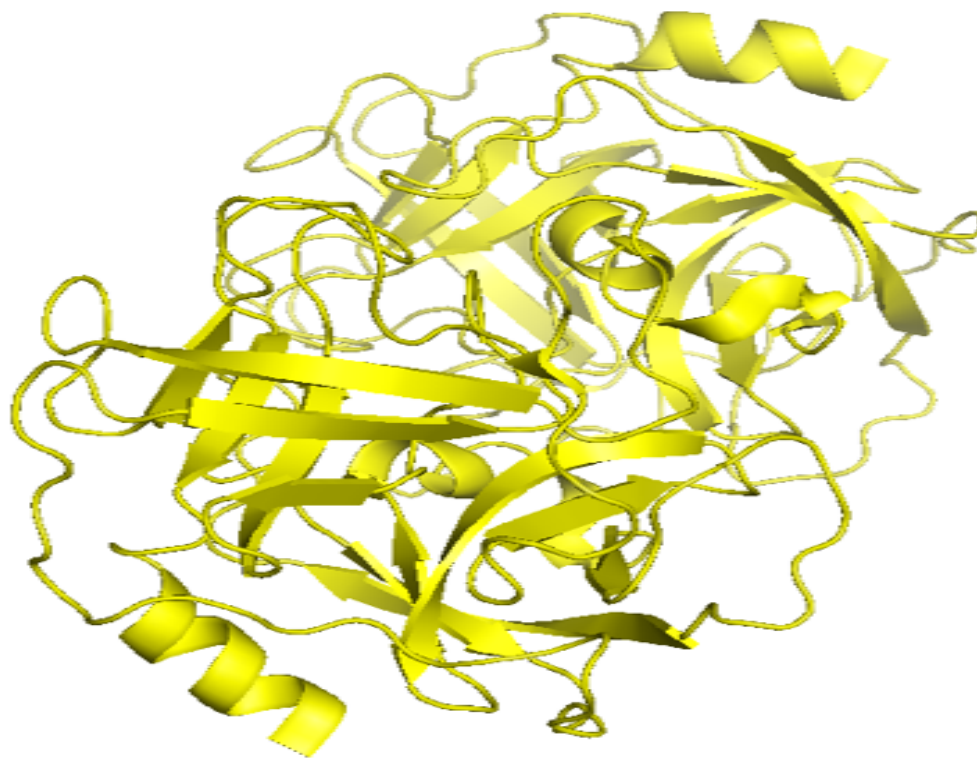
FIGURE 4.7: Given Figure Represented 3d Structures of HNE

FIGURE 4.8: Given Figure Represented 3d Structures of Tyrosinase

4.2   Ligand Selection

Protein data bank contains a large amount of protein ligand complex, especially for the protein target. Therefore, the selection of ligands is based on the best resolution of the structure, the chemical class of the co-crystal ligand bound to the protein structure and the best binding affinity. Conformational selection is a process in which ligand selectively binds to one of these conformers, strengthening it and increasing its population with respect to the total population of the protein is ultimately resulting in the final observed complex.

In concept of ligand-induced shifts the dynamics of protein conformation can be usefully exploited in drug design and drug discovery perspectives [116]. If we need to select a single protein-ligand complex for structural based drug designing, we need to look at the ligand present in the active site. The ligand should bind well to the receptor. Able to adjust the function of proteins (interact with potential residues) and can be able to used as a drug molecules or lead compound. So on the basis of these properties we can select best ligands. If we need to select a single protein-ligand complex for structural based drug designing, we need to look at the ligand present in the active site. The ligand should bind well to the receptor.

PubChem is a public repository for experimental data that identifies the biological activity of small molecules. The structure and other information of ligands were extracted from PubChem database [117]. Bioactive compounds of fenugreek which act as inhibitors were selected as a ligands for the present study and they were represented in Table 4.7.

The selected ligands were alkaloid, flavonoid, phytic acid, 4-hydroxyisoleucin, sapogenin, quercetin, trigonelline, tricin, naringenin and flavonol these selection is on the basis of lispinki rules of fives but there is few more ligands such as Sennoside A, Teprotide, Rutin, Procyanidins and Pectolinarin which can not follow lispinki rules of fives that,s why we excluded these ligands from current research work and these excluded ligands can not considered for further molecular docking analysis. The excluded ligands were shown in table 4.8.

TABLE 4.7: Following Table Represents Name, Molecular Formula, Molecular Weight and Structure of Ligands
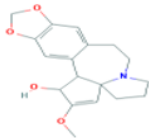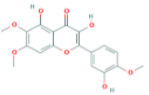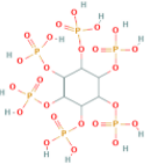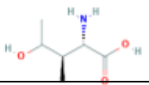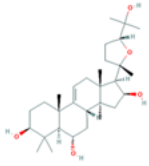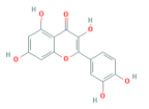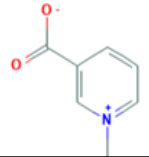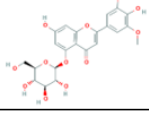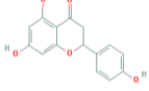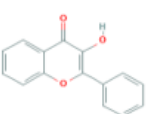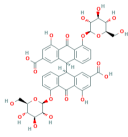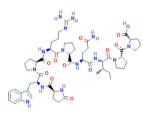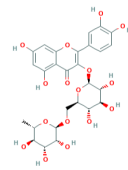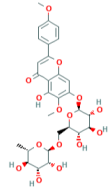
S.No	Name	Molecular Formula	Molecular Weight	Structure
1	Alkaloid	C18H21NO4	315.4 g/mol	
2	Flavonoid	C18H16O8	360.3 g/mol	
3	Phytic acid	C6H18O24 P6	660.04 g/mol	
4	4-Hydroxyisoleucine	C6H13 NO3	147.17 g/mol	
5	Sapogenin	C30H50O5	490.7 g/mol	
6	Quercetin	C15H10O7	302.23 g/mol	
7	Trigonelline	C7H7NO2	137.14 g/mol	
8	Tricin	C17H14O7	330.29 g/mol	
9	Naringenin	C15H12O5	272.25 g/mol	
10	Flavonol	C15H10O3	238.24 g/mol	

TABLE 4.8: Following Table Represents Name, Molecular Formula, Molecular Weight and Structure of Excluded Ligands

S.No	Name	Molecular Formula	Molecular Weight	Structure
1	Sennoside A	C42H38O20	862.7 g/mol	
2	Teprotide	C53H76N14O12	1101.3 g/mol	
3	Rutin	C27H30O16	610.5 g/mol	
4	Procyanidin	C30H26O13	594.5 g/mol	
5	Pectolinarin	C29H34O15	622.6 g/mol	

4.3 Virtual Screening and Toxicity Prediction

PkCSM is an online tool used to find the absorption, distribution, metabolism, excretion and toxicity of a drug [118]. In the present research study, the Lipinski rule has been used for filtration. Applicability of Lipinski rule on ligands was shown in Table 4.9. All ligands follow Lipinski rules of five but except few ligands such as Phytic acid, Sennoside A, Teprotide, Rutin, Procyanidins and Pectolinarin which can not follow lispinki rules of five that,s why we excluded these ligands from

current research work and these excluded ligands can not considered for further research work. According to Lipinski rules;

1. The number of Hydrogen bond donor must be less than 5.

2. The maximum number of Hydrogen bond acceptors must be 10.

3. The log p value must be limited to 5.

4. The molecular weight must be less than 500.

So it means that those ligands which follow Lipinski rules they were considered for molecular docking analysis and those ligands which can not follow Lipinski rules were not considered. Lipinski Rules of Excluded Ligands were shown in Table 4.10.

TABLE 4.9: Applicability of Lipinski Rule on Ligands

S.No	Ligand	logP Value	Molecular Weight	H-Bond Acceptor	H-bond Donor
1	Alkaloid	1.7944	315.4 g/mol	5	1
2	Flavonoid	2.6026	360.3 g/mol	8	3
3	Phytic acid	-3.1326	660.04 g/mol	12	12
4	4-Hydroxyiso leucine	-0.5848	147.17 g/mol	3	3
5	Sapogenin	4.6027	490.7 g/mol	5	4
6	Quercetin	1.988	302.23 g/mol	7	5
7	Trigonelline	-1.1254	137.14 g/mol	2	0
8	Tricin	2.594	330.29 g/mol	7	3
9	Naringenin	2.5099	272.25 g/mol	5	3
10	Flavonol	3.1656	238.24 g/mol	3	1

TABLE 4.10: Lipinski Rules of Excluded Ligands

S.No	Ligand	logP Value	Molecular Weight	H-Bond Acceptor	H-bond Donor
1	Sennoside A	-1.09	862 g/mol	18	12
2	Teprotide	-1.658	1101.3 g/mol	12	10
3	Rutin	-1.6871	610.521 g/-mol	16	10
4	Procyanidin	2.7327	594.525 g/-mol	13	10
5	Pectolinarin	-0.7867	622.576 g/-mol	15	7

4.3.1 Toxicity Prediction

PkCSM is an online tool which provides an integrated platform to rapidly evaluate pharmacokinetic and toxicity properties of a drugs. So this tool is used to find out the toxicity measurements of ligands against Cox-2, mPGES-2, HNE and Tyrosinase which is the target proteins.

4.3.1.1 Alkaloids

Alkaloid is the selected ligand and has inhibitory effect against inflammation. As well the potential activity of the compound also depends on its toxicity. The calculated toxicity values for alkaloids are in acceptable range which increases its activity potential. The toxicity values of alkaloids were shown in Table 4.11.

4.3.1.2 Flavonoid

Flavonoids, a group of natural substances with variable phenolic structures. This bioactive substance which is derived from *Trigonella foenum-graecum* having ability to inhibit key proteins involved in inflammation. The toxicity values of flavonoids were shown in Table 4.12.

TABLE 4.11: Toxicity Values Of Alkaloids

S.No	Model Name	Predicted Values
1	Max.tolerated dose (human)	-0.718 mg/Kg
2	hERG I inhibitor	No
3	hERG II inhibitor	No
4	Oral rat acute toxicity	2.806 mol/Kg
5	Oral rat chronic toxicity	0.313 mg/Kg
6	Hepatoxicity	Yes
7	Skin sensitisation	No
8	t.pyriformis toxicity	0.561 log ug/L
9	Minnow toxicity	1.685 log mM

TABLE 4.12: Toxicity Values of Flavonoids

S.No	Model Name	Predicted Values
1	Max.tolerated dose (human)	0.496 mg/Kg
2	hERG I inhibitor	No
3	hERG II inhibitor	No
4	Oral rat acute toxicity	2.301 mol/Kg
5	Oral rat chronic toxicity	1.972 mg/Kg
6	Hepatoxicity	No
7	Skin sensitisation	No
8	t.pyriformis toxicity	0.313 log ug/L
9	Minnow toxicity	2.341 log mM

4.3.1.3 Phytic Acid

Phytic acid is a unique natural substance found in plant seeds. Phytic acid shows strong inhibitory effect against the spread of pathogenic bacteria. The toxicity values of Phytic acid were shown in Table 4.13.

TABLE 4.13: Toxicity Values of Phytic Acid

S.No	Model Name	Predicted Values
1	Max.tolerated dose (human)	0.435 mg/Kg
2	hERG I inhibitor	No
3	hERG II inhibitor	No
4	Oral rat acute toxicity	2.482 mol/Kg
5	Oral rat chronic toxicity	6.901 mg/Kg
6	Hepatoxicity	No
7	Skin sensitisation	No
8	t.pyriformis toxicity	0.285 log ug/L
9	Minnow toxicity	23.675 log mM

4.3.1.4   4-Hydroxyisoleucine

4-Hydroxyisoleucine is a bioactive compound found in the seeds of *Trigonella foenum-graecum* (fenugreek), which has been used as part of traditional medicine and has shown tremendous effect against infectious diseases. The toxicity values of 4-Hydroxyisoleucine were shown in Table 4.14.

TABLE 4.14: Toxicity Values of 4-Hydroxyisoleucine

S.No	Model Name	Predicted Values
1	Max.tolerated dose (human)	1.277 mg/Kg
2	hERG I inhibitor	No
3	hERG II inhibitor	No
4	Oral rat acute toxicity	2.061 mol/Kg
5	Oral rat chronic toxicity	2.936 mg/Kg
6	Hepatoxicity	No
7	Skin sensitisation	No
8	t.pyriformis toxicity	0.283 ug/L
9	Minnow toxicity	3.272 log mM

4.3.1.5   Sapogenin

Sapogenin are the aglycones, or non-saccharide, portions of the family of natural products known as saponins. Sapogenin have been reported to possess a wide range of anti-inflammatory activities. The toxicity values of Sapogenin were shown in Table 4.15.

TABLE 4.15: Toxicity Values of Sapogenin

S.No	Model Name	Predicted Values
1	Max.tolerated dose (human)	-0.888 mg/Kg
2	hERG I inhibitor	No
3	hERG II inhibitor	No
4	Oral rat acute toxicity	2.395 mol/Kg
5	Oral rat chronic toxicity	1.462 mg/Kg
6	Hepatoxicity	No
7	Skin sensitisation	No
8	t.pyriformis toxicity	0.304 ug/L
9	Minnow toxicity	1.486 mM

4.3.1.6 Quercetin

Quercetin is the most abundant dietary flavonoid derived from plants. This bioactive compound has strong ability to inhibit those proteins which play a vital role in infections. The toxicity values of Quercetin were shown in Table 4.16.

TABLE 4.16: Toxicity Values of Quercetin

S.No	Model Name	Predicted Values
1	Max.tolerated dose (human)	0.438 mg/Kg
2	hERG I inhibitor	No
3	hERG II inhibitor	No
4	Oral rat acute toxicity	2.482 mol/Kg
5	Oral rat chronic toxicity	10.662 mg/Kg
6	Hepatoxicity	No
7	Skin sensitisation	No
8	t.pyriformis toxicity	0.285 log ug/L
9	Minnow toxicity	6.093 log mM

4.3.1.7 Trigonelline

Trigonelline is a chemical compound derived from alkaloid. These chemical compounds prevent inflammation and protects cells in diabetes by inhibiting fetal growth during pregnancy with leptin and insulin. The toxicity values of Trigonelline were shown in Table 4.17.

TABLE 4.17: Toxicity Values of Trigonelline

S.No	Model Name	Predicted Values
1	Max.tolerated dose (human)	0.743 mg/Kg
2	hERG I inhibitor	No
3	hERG II inhibitor	No
4	Oral rat acute toxicity	1.878 mol/Kg
5	Oral rat chronic toxicity	0.454 mg/Kg
6	Hepatoxicity	No
7	Skin sensitisation	No
8	t.pyriformis toxicity	-0.323 log ug/L
9	Minnow toxicity	2.536 log mM

4.3.1.8 Tricin

Tricin is a chemical compound. It is an O-methylated flavone, a type of flavonoid. Tricin has been shown to interfere with TLR4 activation by inhibiting proteins to stimulate inflammatory cascades. The toxicity values of Tricin were shown in Table 4.18.

TABLE 4.18: Toxicity Values of Tricin

S.No	Model Name	Predicted Values
1	Max.tolerated dose (human)	0.351 mg/Kg
2	hERG I inhibitor	No
3	hERG II inhibitor	No
4	Oral rat acute toxicity	2.229 mol/Kg
5	Oral rat chronic toxicity	1.82 mg/kg
6	Hepatoxicity	No
7	Skin sensitisation	No
8	t.pyriformis toxicity	0.329 log ug/L
9	Minnow toxicity	1.754 log mM

4.3.1.9 Naringenin

Naringenin is a flavonoid belonging to flavones subclass. Naringenin can play a protective role by minimizing mucous production during airway inflammation by regulating the production of reactive oxygen species (ROs) and inhibiting NF-kB activity. The toxicity values of Naringenin were shown in Table 4.19.

4.3.1.10 Flavonol

Flavonols are polyphenols belonging to the class of flavonoids. Flavonol use their anti-inflammatory activities by decreasing the production of reactive oxygen species (ROS) and the down-regulation of numerous inflammatory mediators through inhibition of signaling pathways. The toxicity values of Flavonol were shown in Table 4.20.

Table 4.19: Toxicity Values of Naringenin

S.No	Model Name	Predicted values
1	Max.tolerated dose (human)	-0.176 mg/Kg
2	hERG I inhibitor	No
3	hERG II inhibitor	No
4	Oral rat acute toxicity	1.791 mol/Kg
5	Oral rat chronic toxicity	1.944 mg/Kg
6	Hepatoxicity	No
7	Skin sensitisation	No
8	t.pyriformis toxicity	0.369 ug/L
9	Minnow toxicity	2.136 mM

Table 4.20: Toxicity Values Of Flavonol

S.No	Model Name	Predicted values
1	Max.tolerated dose (human)	-0.09 mg/Kg
2	hERG I inhibitor	No
3	hERG II inhibitor	No
4	Oral rat acute toxicity	1.991 mol/Kg
5	Oral rat chronic toxicity	1.582 mg/Kg
6	Hepatoxicity	No
7	Skin sensitisation	No
8	t.pyriformis toxicity	0.699 log ug/L
9	Minnow toxicity	1.205 log mM

4.4 Molecular Docking

Molecular Docking is technique used to estimate the strength of a bond between a ligand and a target protein through a special scoring function and to determine the correct structure of the ligand within the target binding site. The 3D structure of the target proteins and the ligands is taken as the input for docking. It represents a frequently used approach in structure-based drug design since it requires a 3D structure of a target protein.

It can be used to determine the correct structure of the ligand within the target binding site, and to estimate the strength of the binding between the ligand and the target proteins through a specific scoring function.It also helps in the recognition of new small molecular compounds, revealing the essential properties, such as high interaction between binding with target protein having reasonable absorption, distribution, metabolism and excretion, which help in the selection of lead for the target [119].

The docking were performed using Cox-2, m PGES-2, HNE and Tyrosinase proteins and ligands were alkaloid, flavonoid, phytic acid, 4-hydroxyisoleucin, sapogenin, quercetin, trigonelline, tricin, naringenin and flavonol. Ligands with best binding score values with Cox-2, m PGES-2, HNE and Tyrosinase were represented in Table 4.21 A and 4.21 B. To automatically predict binding modes without information about binding sites, we used a user-friendly blind docking web server called CB Dock, which Predicts and estimate a binding site for a given protein and calculate centers and sizes with a novel rotation cavity detection method and perform docking with the popular docking program named Auto dock Vina [120].

CB dock gives 5 best interacting confirmations for each ligand molecule. These confirmations are arranged based on binding affinity and then finest confirmation selection is done on the bases of highest affinity score of protein-ligand interaction. After docking process the dock structure are selected for further analysis, on the basis of docking score,cavity size, Grid map, binding energy, we can select best docked structure.

TABLE 4.21: A: Ligands with Best Binding Score Values with Cox-2, M PGES-2, HNE and Tyrosinase

S.No	Compounds	Alkaloid	Flavonoid	Phytic Acid	4-Hydroxyisoleucine	Sapogenin
1	Binding Score	-6.7	-9.0	-6.8	-5.5	-9.6
2	Cavity size	1232	1232	3377	3795	3377
3	HBD	1	3	12	3	4
4	HBA	5	8	12	3	5
5	logP	1.7499	2.6026	-3.1326	-0.5848	4.6027
6	Molecular Weight g/-mol	315.4 g/-mol	360.3 g/mol	660.04 g/mol	147.17 g/mol	490.7 g/mol
7	Rotatable Bonds	1	4	12	3	2
8	Grid Map	14	14	58	73	58
9	Min-energy Kcl/mol	0	0	0	0	0
10	Max-energy Kcl/mol	1.60E+00	1.60E+00	1.60E+00	1.60E+00	1.60E+00

Continued Table 4.21 B: Ligands with Best Binding Score Values with Cox-2, M PGES-2, HNE and Tyrosinase

S.No	Compounds	Quercetin	Trigonelline	Tricin	Naringenin	Flavonol
1	Binding Score	-9.6	-5.9	-9.3	-8.8	-9.4
2	Cavity Size	4717	4247	3377	1129	4247
3	HBD	5	0	3	3	1
4	HBA	7	2	7	5	3
5	logP	1.988	-1.1254	2.594	2.5099	3.1656
6	Molecular Weight g/mol	302.23 g/mol	137.14 g/mol	330.29 g/-mol	272.25 g/mol	238.24 g/mol
7	Rotatable Bonds	1	1	3	1	1
8	Grid Map	36	20	58	-38	20
9	Min-energy Kcl/mol	0	0	0	0	0
10	Max-energy Kcl/mol	1.60E+00	1.60E+00	1.60E+00	1.60E+00	1.60E+00

4.5 Interaction of Ligands and Target Protein

The interaction of the active pockets of the ligand and the protein are calculated for the interpretation of docking results. Two types of interactions are studied; hydrogen bonding and hydrophobic bonding interaction. Using Ligplot plus (version v.1.4.5) the protein ligand interactions were studied [121]. By using Ligplot plus the interaction of active confirmation of ligands and the target protein has been identified. The saved conformations for ligand receptor complex of each molecule are analyzed in detail. This software automatically generates schematic diagrams of the protein-ligand interactions of the given ligands in the PDB file. The docked files are uploaded in PDB format to get hydrogen and hydrophobic bonding. Hydrogen bonding and hydrophobic interactions of active ligands were shown in Table 4.22. The ligand Alkaloid made 2 hydrogen bonds and 2 hydrophobic interactions. The residues involved in hydrogen bonding are ASN and GLU as show in Figure 4.9. The ligand Flavonoid made 3 hydrogen bonds and 11 hydrophobic interactions. The residues involved in hydrogen bonding are ASN, CYS and GLU were shown in Figure 4.10. The Phytic acid made 4 Hydrogen bonds and 10 hydrophobic interactions. The residues involved in hydrogen bonding are ASN, GLY, GLY and GLN were shown in Figure 4.11. The 4-Hydroxyisoleucine made 5 Hydrogen bonds and 6 hydrophobic interactions. The residues involved in hydrogen bonding are GLY, MET, SER, TYR and VAL were shown in Figure 4.12. The Sapogenin made 2 Hydrogen bonds and 15 hydrophobic interactions. The residues involved in hydrogen bonding are ASN and GLY were shown in Figure 4.13. The Quercetin made 5 Hydrogen bonds and 6 hydrophobic interactions. The residues involved in hydrogen bonding are ALA, CYS, GLY, GLU and PRO were shown in Figure 4.14. The Trigonelline made 2 Hydrogen bonds and 8 hydrophobic interactions. The residues involved in hydrogen bonding are GLN and GLN which were shown in Figure 4.15. The Tricin made 4 Hydrogen bonds and 9 hydrophobic interactions. The residues involved in hydrogen bonding are ASN, ASN, CYS and GLY were shown in Figure 4.16. The Naringenin made 1 Hydrogen bond and 6 hydrophobic interactions. The residue involved in hydrogen bonding

is CYS and was shown in Figure 4.17. The Flavonol made 1 Hydrogen bond and 12 hydrophobic interactions. The residue involved in hydrogen bonding is ASP which was shown in Figure 4.18. A significant number of hydrophobic and hydrogen bond interactions are observed between the ten ligands and the four target proteins. Ligand-receptor complex shows strong hydrogen bonding, hydrophobic interactions and van der Waal forces [122].
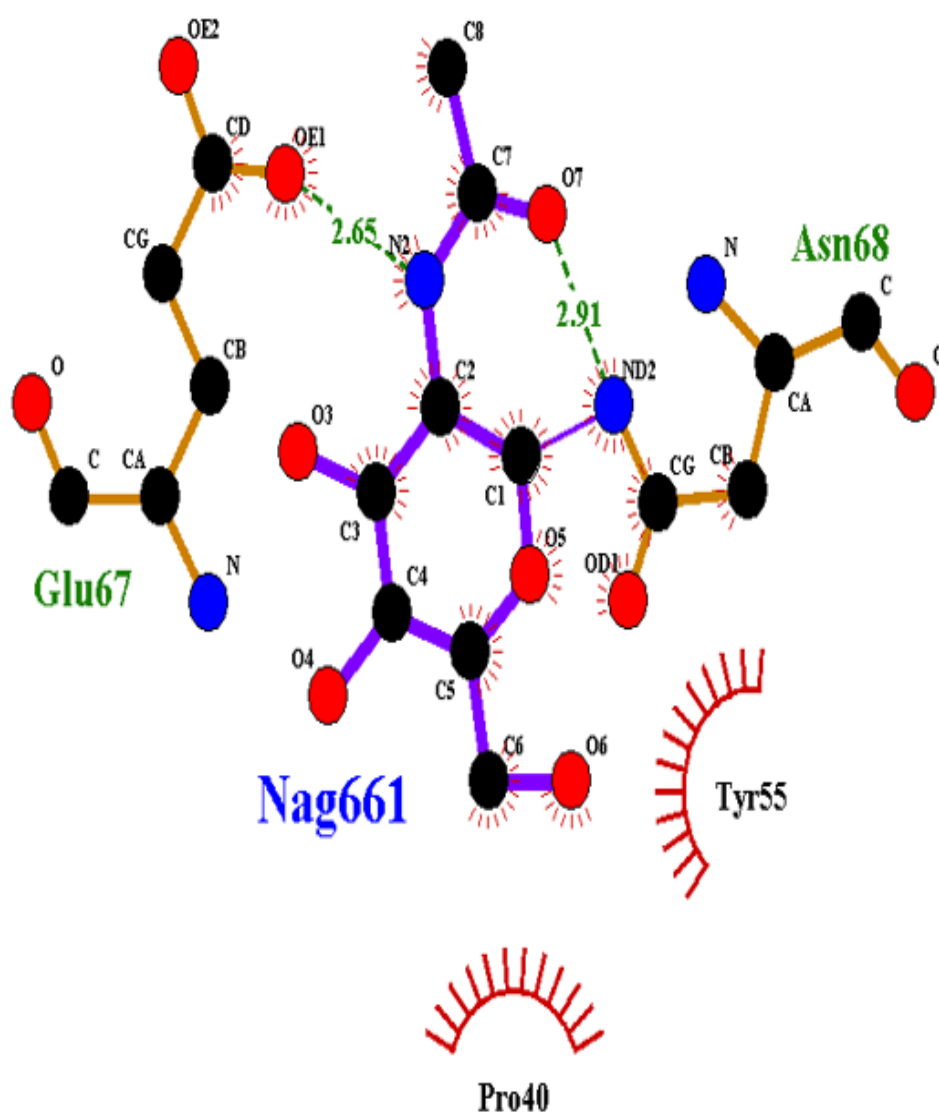
FIGURE 4.9: 2D Representation of Docked Complex Alkaloid Using Ligplot-Plus
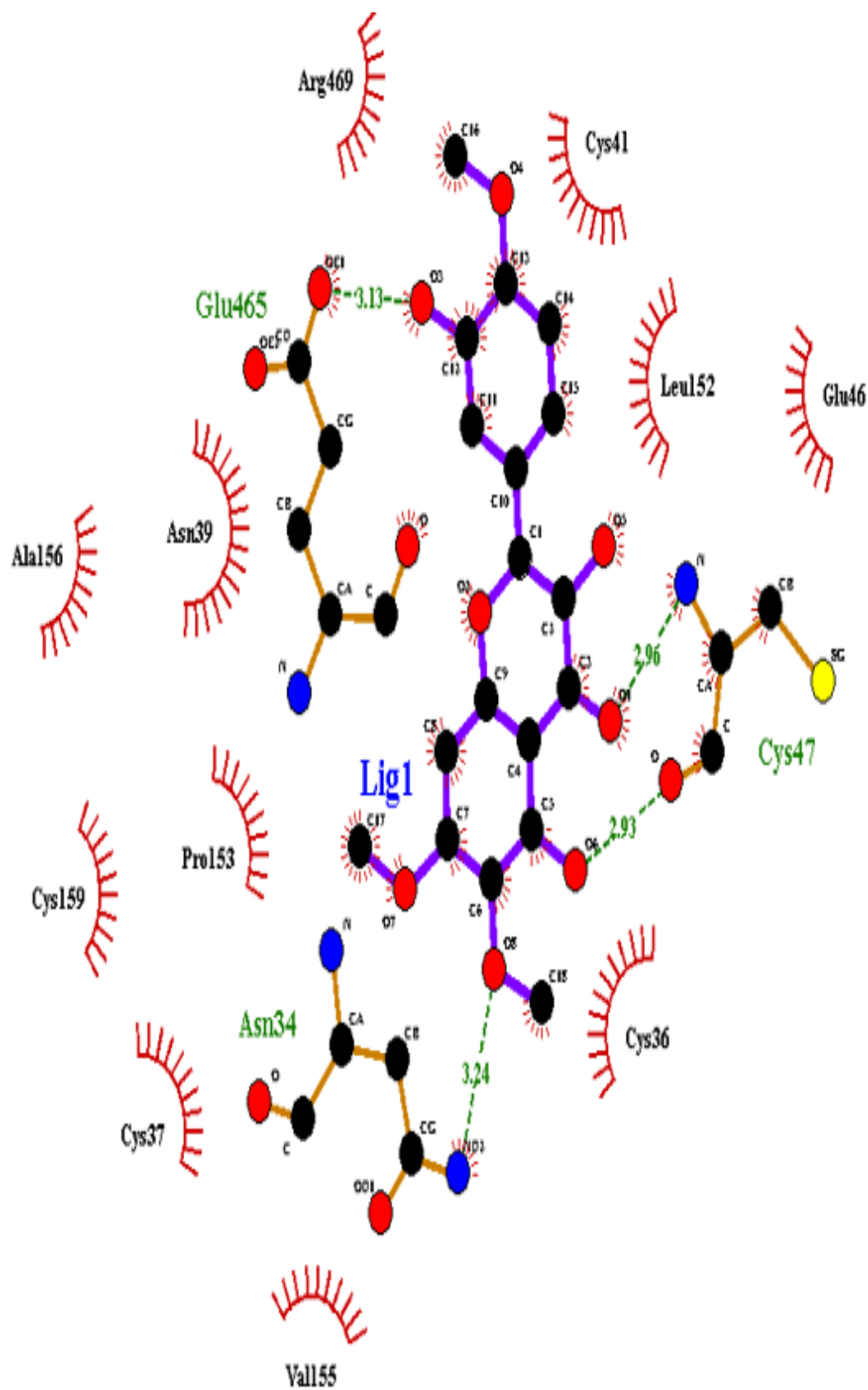
FIGURE 4.10: 2D Representation of Docked Complex Flavonoid Using Ligplot-Plus
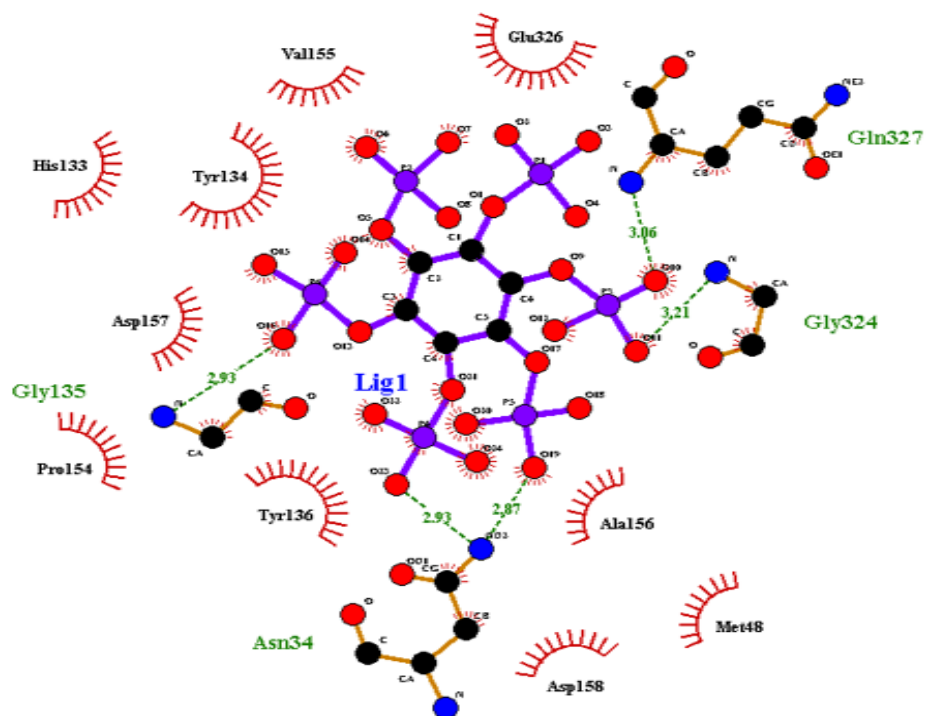
FIGURE 4.11: 2D Representation of Docked Complex Phytic Acid Using Ligplot-Plus
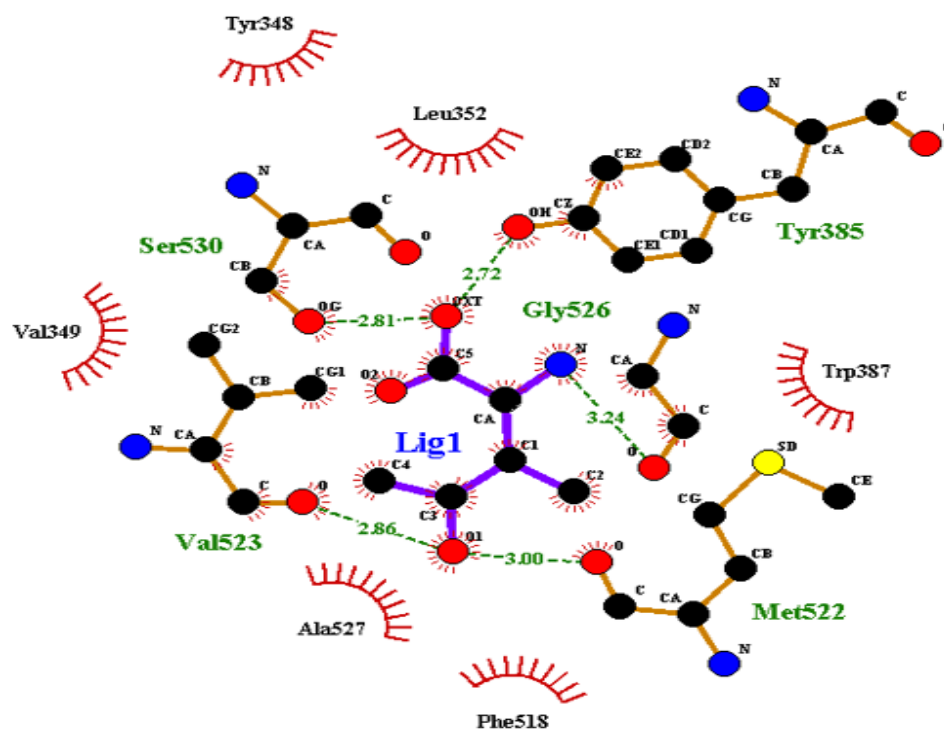
FIGURE 4.12: 2D Representation of Docked Complex 4-Hydroxyisoleucine Using Ligplot-Plus
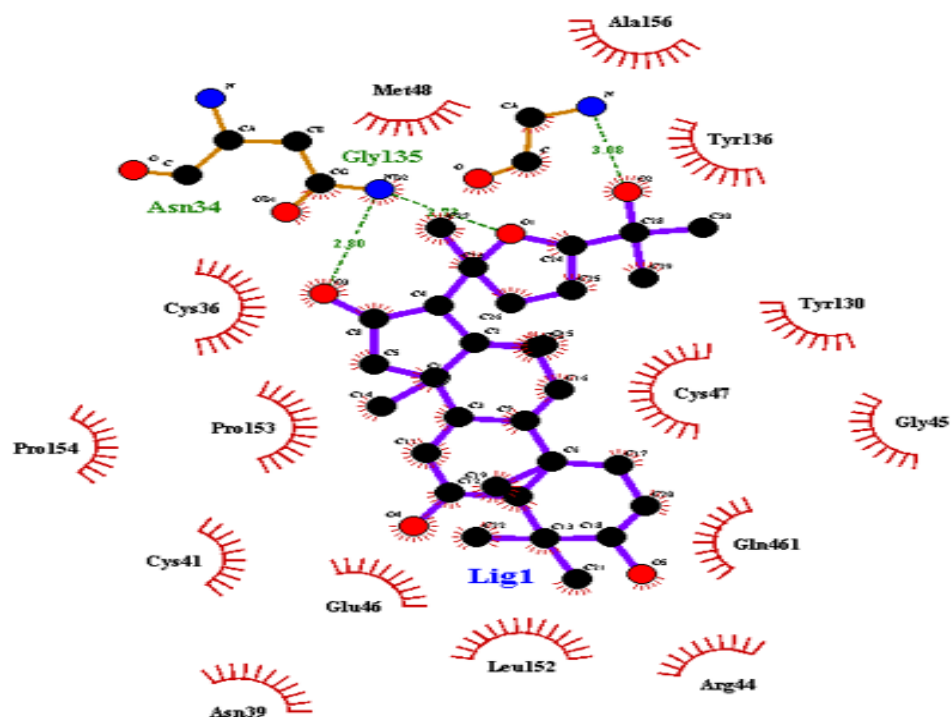
FIGURE 4.13: 2D Representation of Docked Complex Sapogenin Using Ligplot-Plus
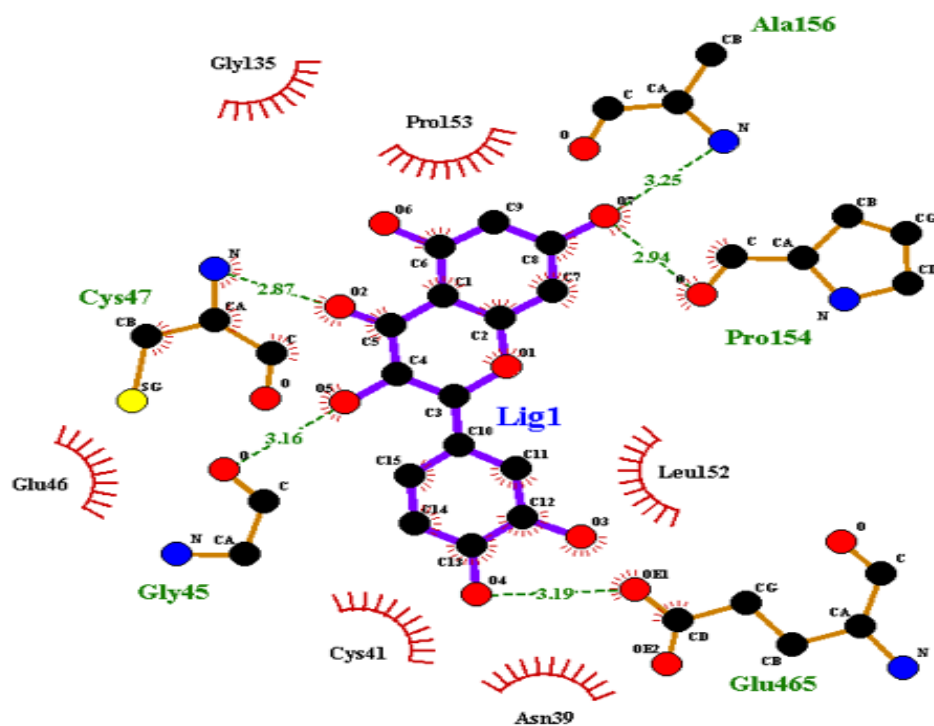
FIGURE 4.14: 2D Representation of Docked Complex Quercetin Using Ligplot-Plus
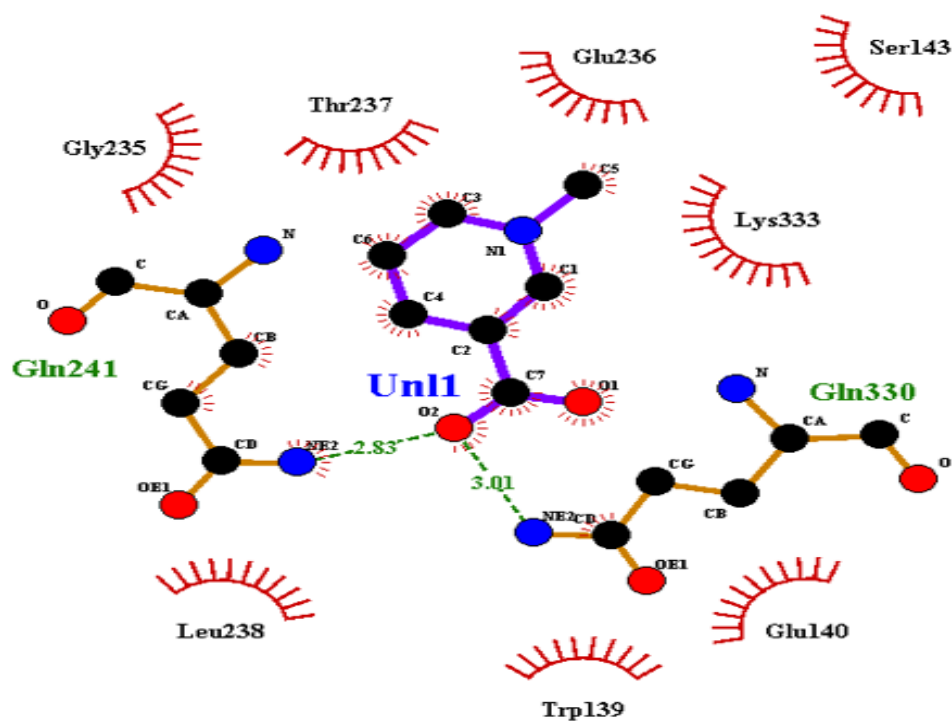
FIGURE 4.15: 2D Representation of Docked Complex Trigonelline Using Ligplot-Plus
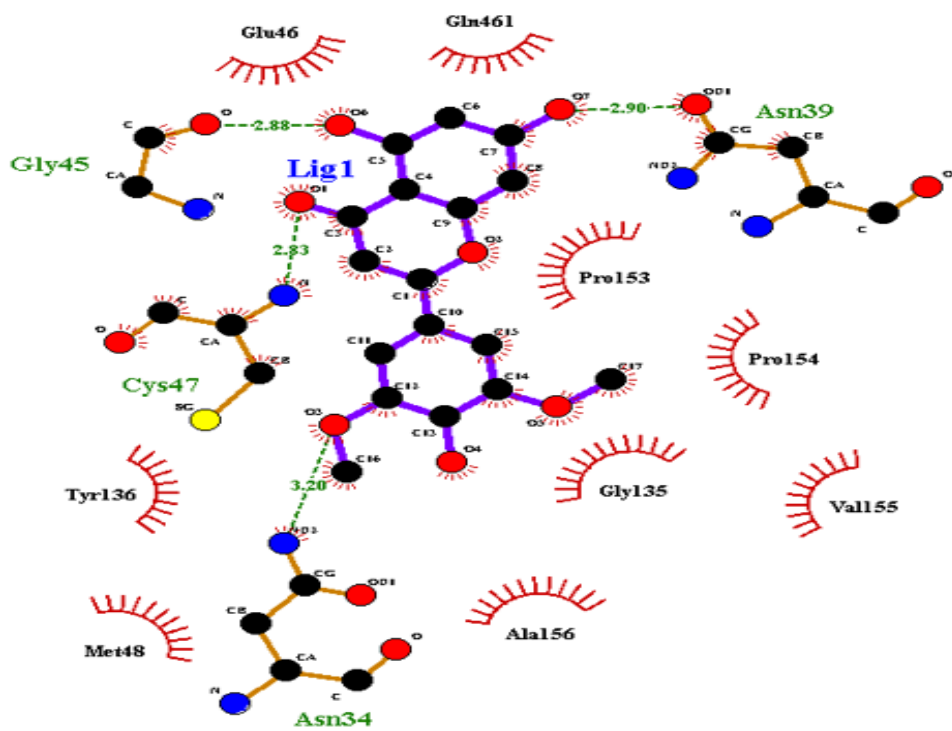
FIGURE 4.16: 2D Representation of Docked Complex Tricin Using Ligplot-Plus
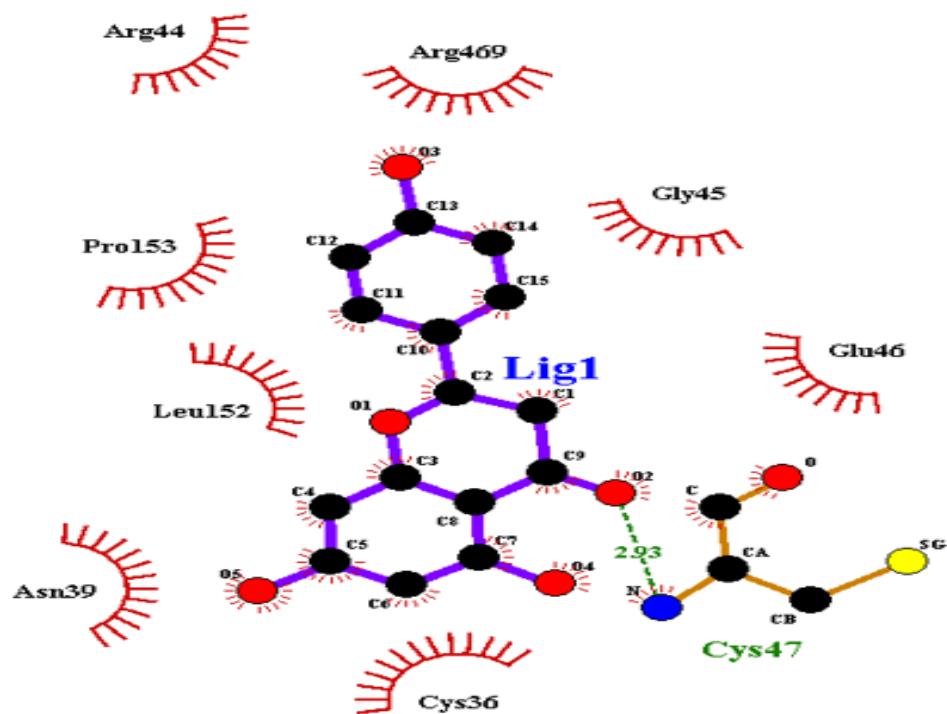
FIGURE 4.17: 2D Representation of Docked Complex Naringenin Using Ligplot-Plus
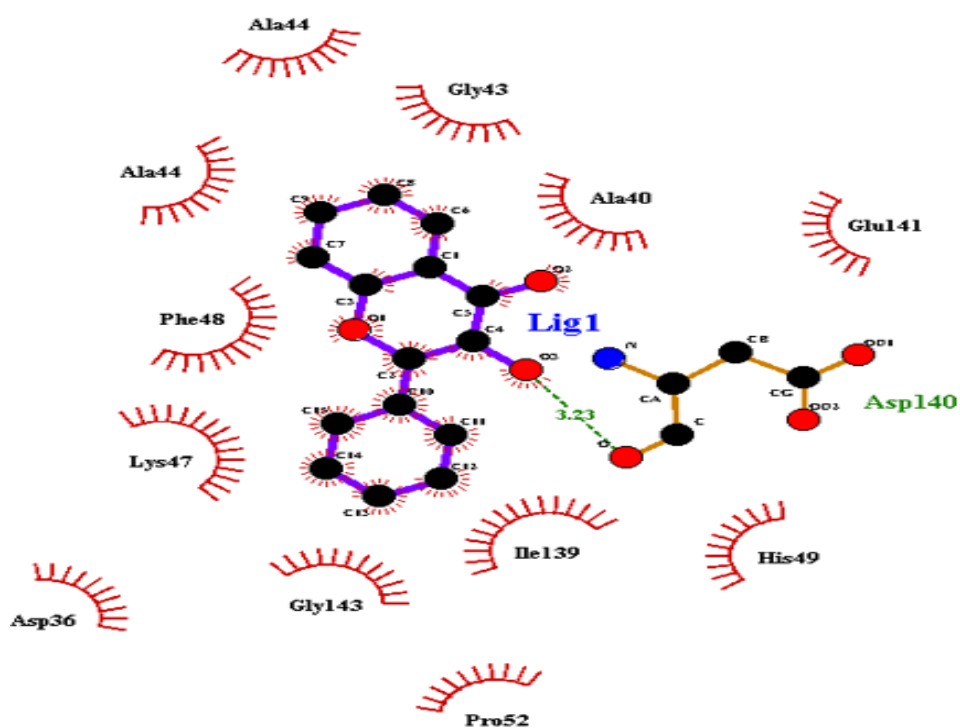
FIGURE 4.18: 2D Representation of Docked Complex Flavonol Using Ligplot-Plus

TABLE 4.22: Active Ligand Showing Hydrogen and Hydrophobic Interactions

S.No	Ligand Name	Binding Energy	No. of HBs	Hydrogen Bonding Amino Acids	Distance	Hydrophobic Bonding
1	Alkaloid	-6.7	2	ND:ASN: O N:GLU:OE	2.91 2.65	Tyr55 Pro40
2	Flavonoid	-9.0	3	ND-ASN:O N:ARG:O OC1:ASP:O	3.24 2.96 3.13	Asn39 Ala156 Arg469 Cys36 Cys37 Cys41 Cys159 Glu46 Leu152 Pro153 Val155
3	Phytic Acid	-6.8	4	OD-ASN:O N-GLY:O N-GLY:O	2.87 3.21 3.06	Ala156 Asp157 Asp158

Continued Table 4.22: Active Ligand Showing Hydrogen and Hydrophobic Interactions.

| S.No | Ligand Name | Binding Energy | No. of HBs | Hydrogen Bonding | | Hydrophobic Bonding |
				Amino Acids	Distance	
				N-GLN:O	2.93	Glu326
						His133
						Met48
						Pro154
						Tyr134
						Tyr136
						Val155
4	4-Hydroxyisoleucine	-5.5	5	N-GLY:O	3.24	Ala527
				N-MET:O	3.00	Leu352
				N-SER:OG	2.81	Tyr348
				N-TYR:OH	2.72	Trp387
				N-VAL:O	2.86	Phe518
						Val349

Continued Table 4.22: Active Ligand Showing Hydrogen and Hydrophobic Interactions.

S.No	Ligand Name	Binding Energy	No. of HBs	Hydrogen Bonding Amino Acids	Distance	Hydrophobic Bonding
5	Sapogenin	-9.6	2	ND-ASN:O	2.80	Asn39
				ND-GLY:O	2.72	Arg44
						Ala156
						Cys36
						Cys41
						Cys47
						Gly45
						Glu46
						Gln461
						Leu152
						Met48
						Pro153
						Pro154
						Tyr130
						Tyr136
6	Quercetin	-9.6	5	N-ALA:O	3.25	Asn39
				N-CYS:O	2.87	Cys41

Continued Table 4.22: Active Ligand Showing Hydrogen and Hydrophobic Interactions.

S.No	Ligand Name	Binding Energy	No. of HBs	Hydrogen Bonding Amino Acids	Distance	Hydrophobic Bonding
				N-GLU:OE	3.19	Glu46
				N-GLY:O	3.16	Gly153
				N-PRO:O	2.94	Pro153
						Leu152
7	Trigonelline	-5.9	2	NE-GLN:O	2.83	Glu140
				NE-GLN:O	3.01	Gly235
						Glu236
						Leu238
						Lys333
						Ser143
						Trp139
						Thr237

Continued Table 4.22: Active Ligand Showing Hydrogen and Hydrophobic Interactions.

S.No	Ligand Name	Binding Energy	No. of HBs	Hydrogen Bonding Amino Acids	Distance	Hydrophobic Bonding
8	Tricin	-9.3	4	ND-ASN:O	3.20	Ala156
				ND-ASN:O	2.90	Glu46
				N-CYS:O	2.83	Gly135
				N-GLY:O	2.88	Gln461
						Met48
						Pro153
						Pro154
						Tyr136
						Val155
9	Naringenin	-8.8	1	N-CYS:O	2.93	Asn39
						Arg44
						Arg469
						Cys36
						Gly45
						Glu46
						Leu152
						Pro153

Continued Table 4.22: Active Ligand Showing Hydrogen and Hydrophobic Interactions.

S.No	Ligand Name	Binding Energy	No. of HBs	Hydrogen Bonding Amino Acids	Distance	Hydrophobic Bonding
10	Flavonol	-9.4	1	N-ASP:O	3.23	Asp36
						Ala40
						Ala44
						Ala44
						Ile139
						Gly43
						Glu141
						Gly143
						His49
						Lys47
						Phe48
						Pro52

4.6 ADME Properties of Ligands

ADME properties of ligands extracted from pkCSM online tool. Toxicity provides insights into the nature of ligands, which must be considered before designing a drug. To use a compound as a chemotherapeutic agent, it must first be tested for toxicity. PkCSM is used to find the toxicity and ADME properties of the drug. This server takes SMILES as input. The dragged server values are as follow.

4.6.1 Absorption

In pharmacology (specifically pharmacokinetics), the transfer of a drug from the bloodstream into the tissues is called absorption. So the chemical composition of a drug, as well as the environment into which a drug is placed, work together to determine the rate and extent of drug absorption.

For drug absorption it is necessary, that a drug must cross cellular barriers for example epithelial or endothelial cells, etc. Only a few drugs move across cellular barriers in an "active" way; that is, a way that requires energy (ATP) and moves the drug from an area of low concentration to an area of higher On the other hand, most drugs cross cellular barriers via passive diffusion; that is, drugs simply move from an area of higher concentration to an area of lower concentration by diffusing through cell membranes.

This type of drug movement does not require any energy expenditure, but will be influenced by the size of the drug and the solubility of the drug. Absorption properties of ligands were shown in Table 4.23 and 4.24 respectively.

TABLE 4.23: Absorption Properties of Ligands

S.No	Ligands	Alkaloid	Flavonoid	Phytic acid	4-Hydroxyisoleucine	Sapogenin
1	Water solubility	-2.721 mol/L	-3.178 mol/L	2.797 mol/L	-2.888 mol/L	-5.433 mol/L
2	Caco2 permeability	1.45 cm/S	0.367 cm/S	1.953 cm/S	0.505 cm/S	1.055 cm/S
3	Intestinal absorption (human)	90.091 %	92.645 %	66.375 %	57.573 %	95.08 %
4	Skin Permeability	-3.248 log Kp	-2.735 log Kp	2.735	-2.735 log Kp	-3.702 log Kp
5	P-glycoprotein substrate	No	Yes	Yes	No	Yes
6	P-glycoprotein I inhibitor	No	No	No	No	Yes
7	P-glycoprotein II inhibitor	No	Yes	No	No	No

TABLE 4.24: Absorption Properties of Ligands

S.No	Ligands	Quercetin	Trigonelline	Tricin	Naringenin	Flavonol
1	Water solubility	-2.925 mol/L	-1.931 mol/L	-3.276 mol/L	3.224 mol/L	-3.683 mol/L
2	Caco2 permeability	-0.229 cm/S	1.124 cm/S	0.12 cm/S	1.029 cm/S	1.263 cm/S
3	Intestinal absorption (human)	77.207 %	96.44 %	89.713 %	91.31 %	94.776 %
4	Skin Permeability	-2.735 log Kp	-2.736 log Kp	-2.735 log Kp	2.742 log Kp	-2.775 log Kp
5	P-glycoprotein substrate	Yes	Yes	Yes	Yes	Yes
6	P-glycoprotein I inhibitor	No	No	No	No	No
7	P-glycoprotein II inhibitor	No	No	No	No	No

4.6.2   Distribution

Distribution in pharmacology is a branch of pharmacokinetics which deals with the movement of drug within the body from one location to another location. When a drug enters the systemic circulation by absorption or direct administration, it must be distributed into interstitial and intracellular fluids. The distribution of ligands were shown in Table 4.25 and 4.26 respectively.

TABLE 4.25: Distribution of Ligands

S.No	Ligands	Alkaloid	Flavonoid	Phytic acid	4-Hydroxyisoleucine	Sapogenin
1	VDss (human)	0.831 L/Kg	0.313 L/Kg	0.011 L/Kg	-0.539 L/Kg	0.258 L/Kg
2	Fraction unbound (human)	0.396 Fu	0.053 Fu	0.381 Fu	0.474 Fu	0.151 Fu
3	BBB permeability	0.19 log BB	-1.244 log BB	-8.285 log BB	-0.564 log BB	-0.514 log BB
4	CNS permeability	-2.329 log PS	-3.262 log PS	-9.599 log PS	-3.489 log PS	-2.995 log PS

TABLE 4.26: Distribution of Ligands

S.No	Ligands	Quercetin	Trigonelline	Tricin	Naringenin	Flavonol
1	VDss (human)	1.559 L/Kg	-0.758 L/Kg	0.798 L/Kg	-0.015 L/Kg	0.214 L/Kg
2	Fraction unbound (human)	0.206 Fu	0.857 Fu	0.084 Fu	0.064 Fu	0.151 Fu
3	BBB permeability	-1.098 log BB	-0.234 log BB	-1.115 log BB	-0.578 log BB	0.462 log BB
4	CNS permeability	-3.065 log PS	-2.739 log PS	-3.411 log PS	-2.215 log PS	-1.733 log PS

4.6.3 Metabolism

Metabolism is the process of converting one compound into another with the help of enzymes. Mostly metabolism occurs in the plasma of blood, liver, intestine and lungs. Generally, the metabolic process will convert the drug into a more water-soluble compound by increasing its polarity. Metabolic properties of ligands were shown in Table 4.27 and 4.28 respectively.

TABLE 4.27: Metabolic Properties of Ligands

S.No	Ligands	Alkaloid	Flavonoid	Phytic acid	4-Hydroxyisoleucine	Sapogenin
1	CYP2D6 substrate	No	No	No	No	No
2	CYP3A4 substrate	Yes	No	No	No	Yes
3	CYP1A2 inhibitior	No	Yes	No	No	No
4	CYP2C19 inhibitior	No	No	No	No	No
5	CYP2C9 inhibitior	No	No	No	No	No
6	CYP2D6 inhibitior	No	No	No	No	No
7	CYP3A4 inhibitior	No	No	No	No	No

TABLE 4.28: Metabolic Properties of Ligands

S.No	Ligands	Quercetin	Trigonelline	Tricin	Naringenin	Flavonol
1	CYP2D6 substrate	No	No	No	No	No
2	CYP3A4 substrate	No	No	No	No	Yes
3	CYP1A2 inhibitior	Yes	Yes	Yes	Yes	Yes
4	CYP2C19 inhibitior	No	No	Yes	No	Yes
5	CYP2C9 inhibitior	No	No	No	No	No
6	CYP2D6 inhibitior	No	No	No	No	No
7	CYP3A4 inhibitior	No	No	No	No	No

4.6.4 Excretion

The organs involved in drug excretion are the kidneys, which play important role in excretion (renal excretion) and the liver (biliary excretion). Other organs may also be involved in excretion, such as the lungs for volatile or gaseous agents. Drugs can also be excreted in sweat, saliva and tears. Excretory properties of ligands were shown in Table 4.29 and 4.30.

TABLE 4.29: Excretory Properties of Ligands

S.No	Ligands	Alkaloid	Flavonoid	Phytic acid	4-Hydroxyisoleucine	Sapogenin
1	Total Clearance	1.032 ml/Kg	0.549 ml/Kg	-0.218 ml/Kg	0.35 ml/Kg	1.218 ml/Kg
2	Renal OCT2 substrate	No	No	No	No	Yes

TABLE 4.30: Excretory Properties of Ligands

S.No	Ligands	Quercetin	Trigonelline	Tricin	Naringenin	Flavonol
1	Total Clearance	0.407 ml/Kg	0.378 ml/Kg	0.62 ml/Kg	0.06 ml/Kg	-21.759 ml/Kg
2	Renal OCT2 substrate	No	No	No	No	No

4.7   Lead Compound Identification

Sapogenin is identified as the lead compound which shows the best binding score, hydrogen bonding and pharmacokinetic properties. The toxicity measurements by online tool pkCSM. Sapogenin has been chosen as the lead compound because it is the most active compound for the inhibition of COX2, mPGES-2, HNE and Tyrosinase.

4.8   Anti-Inflammatory Drug Identification

The most popular and effective drugs used for the inflammation were listed in the Table 4.31 along with their mechanism of action. So these drugs show great results against inflammation. KEGG and Drug Bank databases are used for these drugs identification along with their mechanism of action.

TABLE 4.31: Drugs And Their Mechanism of Action

S.No	Drugs	Mechanism of action	References
1	Aspirin (Acetosal)	Aspirin stops prostaglandin synthesis. It is non-selective for COX-1 and COX-2 enzymes.	[123]
2	Ibuprofen (2-hydroxyibuprofen)	The primary mechanism of action of ibuprofen is the inhibitory, reversible inhibition of cyclooxygenase enzymes Cox-1 and Cox-2 (coded by PTGS-1 and PTGS-2, respectively).	[124]
3	Paracetamol (Acetaminophen)	Paracetamol has a central analgesic effect that is mediated by activating the descending serotonergic pathways. There is debate about its primary site of action, which may inhibit prostaglandin (PG) synthesis.	[125]
4	Celebrex (Etoricoxib)	The mechanism of action of Celebrex is due to the inhibition of the selection of cyclooxygenase-2 (COX-2), which is an integral part of the pain and inflammation pathway responsible for prostaglandin synthesis.	[126]
5	Diclofenac (Voltaren)	The mechanism of action of Diclofenac is to inhibit cyclooxygenase-1 and -2, because these enzymes are responsible for production of prostaglandin (PG) G2 which is the precursor to other PGs.	[127]
6	Naproxen (Aflaxen)	Naproxen acts by inhibiting the COX-1 and COX-2 and other enzymes as well which involved in inflammation.	[128]

4.9 Selection of Anti-Inflammatory Drugs

The selection of most efficient anti-inflammatory drug is based on the physiochemical, ADMET properties along mechanism of action with side effects. For physiochemical properties PubChem online database is used and for ADMET properties of drugs pkCSM online tool is used. Mechanism of action is identified through Drug Bank and KEGG databases. Selected anti-inflammatory drugs and their properties were shown in Table 4.32 and 4.33.

TABLE 4.32: Given Table Shows Us Drugs and Their Properties

S.No	Properties	Aspirin	Ibuprofen	Paracetamol
1	Chemical formula	C9H8O4	C13H18O2	C8H9NO2
2	Absorption	Intestinal absorption of Aspirin occurs at a much faster rate.	Approximately take 2 hours	2–2.5 hours
3	Water solubility mg/ml	-1.868	-3.696	-1.661
4	logP	1.3101	3.0732	1.3506
5	H-bond donor	1	1	2
6	H-bond Acceptor	3	1	2
7	Bioavailability	1	1	1
8	Polarizability	17.1 Å3	23.76 Å3	15.52 Å3
9	ADMET probability	0.9645	0.9927	0.9921
10	Side Effects	· Rash, GI ulcerations, Abdominal pain, Upset stomach, Heartburn, Drowsiness, headache, cramping, nausea,gastritis and Bleeding.	Headache, Dizziness, Drowsiness, Fatigue and restless sleep, Thirst and sweating.	Allergic reaction, Flushing, Low blood pressure and a Fast heartbeat, Blood disorders, liver and kidney damage.

TABLE 4.33: Given Table Shows Us Drugs and Their Properties

S.No	Properties	Celebrex	Diclofenac	Naproxen
1	Chemical formula	C17H14F3N3O2S	C14H11Cl2NO2	C14H14O3
2	Absorption	Rapid	Take 4.5 hours	Take 5 hours
3	Water solubility mg/ml	-4.45	-3.863	-3.144
4	logP	3.51392	4.3641	3.0365
5	H-bond donor	1	2	1
6	H-bond Acceptor	4	2	2
7	Bioavailability	1	1	1
8	Polarizability	35.2 Å3	27.93 Å3	24.81 Å3
9	ADMET probability	1.00	0.9548	0.9948
10	Side Effects	Stomach pain, Constipation, Diarrhea, Heartburn, Nausea, Vomiting and Dizziness.	Headaches, dizziness, Stomach pain, Diarrhea and Rashes.	Headache, Ringing in the ears, Changes in vision, Tiredness, Drowsiness, Dizziness and Rashes.

4.9.1 Celebrex Drug

Celecoxib is a Cox-2 selective inhibitor and is a nonsteroidal anti-inflammatory drug (NSAID). Celecoxib block the cyclooxygenase 2 (COX-2) and other enzymes activity as well, and it is used to treat pain and inflammation [129]. It is marketed by Pfizer under the name Celebrex, and was approved by the Food and Drug Administration (FDA) in 1998. 2D structure of Celebrex drug is shown in Figure 4.19. The Food and Drug Administration panel concluded that a prescription painkiller that has been under decade seems to be safer than previously thought. Celecoxib, a drug sold by Pfizer under the name Celebrex, has no higher risk of heart attack and stroke than other widely used painkillers. It is usefulness in relieving pain, swelling and improving the physical function of the body [130]. It is a generic drug sold in US, UK, Spain, Germany and mostly in Asia to treat pain and inflammation. Physiochemical properties of Celebrex were shown in Table 4.34.
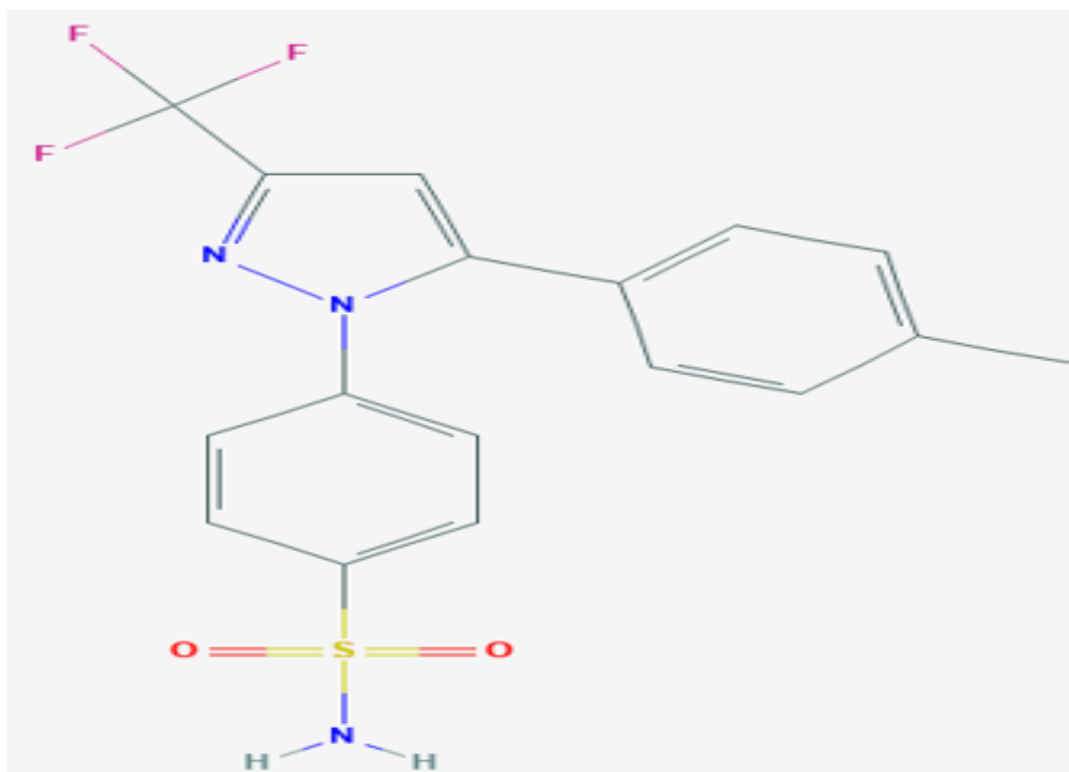
FIGURE 4.19: 2D Structure of Celebrex Drug from PubChem Database

<div align="center">Table 4.34: Physiochemical Properties of Celebrex</div>

logP Value	Rotatable Bonds	H-bond Acceptor	H-bond Donor	Molecular Formula	Molecular Weight
3.51392	3	4	1	C17H14F3N3O2S	381.379

4.10 Drug ADMET Properties

The ADMET properties of the selected drug (Celebrex) were identified through pkCSM online prediction tool.

4.10.1 Toxicity Prediction of Selected Drug

The toxicity value of selected drug Celebrex were given below in Table 4.35.

<div align="center">Table 4.35: Toxicity Values of Celebrex</div>

S.No	Model Name	Predicted values
1	Max.tolerated dose(human)	0.178 mg/Kg
2	hERG I inhibitor	No
3	hERG II inhibitor	No
4	Oral rat acute toxicity	1.975 mol/Kg
5	Oral rat chronic toxicity	1.526 mg/Kg
6	Hepatoxicity	Yes
7	Skin sensitization	No
8	t.pyriformis toxicity	0.43 ug/L
9	Minnow toxicity	0.86 log mM

4.10.2 Absorption Properties

The absorption properties of selected drug Celebrex were given below in Table 4.36.

Table 4.36: Absorption Values of Celebrex

S.No	Model Name	Value
1	Water solubility	-4.45 mol/L
2	Caco2 permeability	0.839 cm/S
3	Intestinal absorption (human)	92.995 %
4	Skin Permeability	-2.692 log Kp
5	P-glycoprotein substrate	Yes
6	P-glycoprotein I inhibitor	Yes
7	P-glycoprotein II inhibitor	Yes

4.10.3   Distribution Properties

The distribution properties of selected drug Celebrex were given below in Table 4.37.

Table 4.37: Distribution Properties of Selected Drug Celebrex

S.No	Model Name	Value
1	VDss (human)	-0.273 L/Kg
2	Fraction unbound (human)	0.133 Fu
3	BBB permeability	-0.931 log BB
4	CNS permeability	-2.052 log PS

4.10.4   Metabolic Properties

The metabolic properties of selected drug Celebrex are given below in Table 4.38.

Table 4.38: Metabolic Properties of Selected Drug Celebrex

S. No	Model Name	Predicted Value
1	CYP2D6 substrate	No
2	CYP3A4 substrate	Yes
3	CYP1A2 inhibitor	Yes
4	CYP2C19 inhibitor	Yes
5	CYP2C9 inhibitor	Yes
6	CYP2D6 inhibitor	No
7	CYP3A4 inhibitor	Yes

4.10.5 Excretion Properties

The excretion properties of selected drug Celebrex were given below in Table 4.39.

TABLE 4.39: Excretion Properties of Selected Drug Celebrex

S.No	Model Name	Predicted Value
1	Total Clearance	0.435 ml/Kg
2	Renal OCT2 substrate	No

4.11 Celebrex Mechanism of Action

Celebrex is the selected drug which was selected through comparing physiochemical and ADMET properties with other identified drugs. Celecoxib, it also works by blocking synthesis of Prostaglandins involved in pain, fever and inflammation [131]. Cox-2 catalyzes arachidonic acid by converting prostaglandin G1& G2 (PGE1 & PGE2) to prostaglandin H1& H2 (PGH1 &PGH2). In the Cox-2 catalyzed pathway, PGH2 is a precursor to prostaglandin E2 (PGE2) and I2 (PGI2). PGE2 causes pain, fever, swelling and redness of the skin. Celecoxib binding to the upper portion of the active site, it forms an antagonist of COX-2, preventing its substrate, arachidonic acid, from entering the active site.
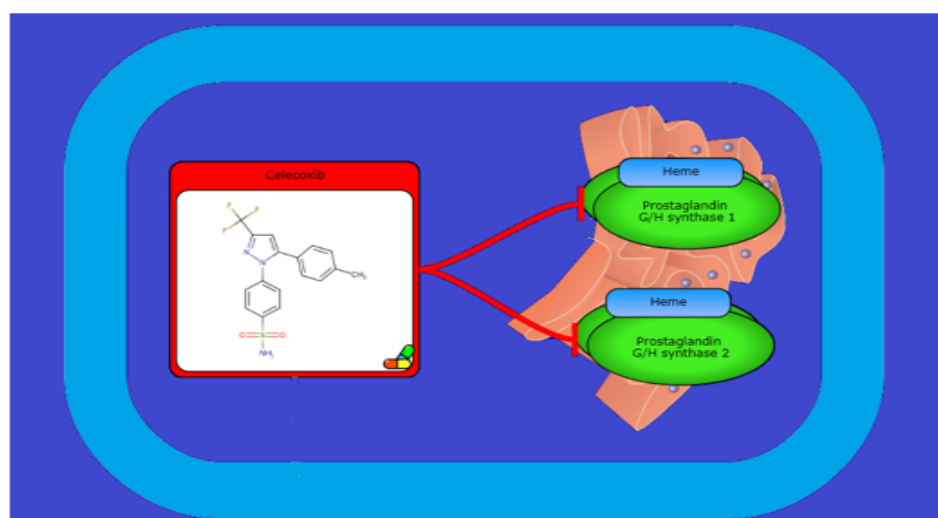
FIGURE 4.20: Celebrex Drug Mechanism of Action Through Drug Bank

4.12   Celebrex Effects on Body

Celebrex which having both positive and negative effects on the body. Firstly being used as infectious disease treatment, it can also reduce pain, fever and inflammation and use as a painkiller. Celebrex increases the risk of bleeding due to ulcers in stomach or intestine, which can be deadly. It can happen at any time without warning signs. If you are older, you are at higher risk for these problems. By stopping prostaglandin synthesis, non-steroidal anti-inflammatory drugs (NSAIDs) damage the mucus in the gastrointestinal tract, causing ulceration and ulcer complications [132]. Celebrex has a lower risk of causing ulceration than other NSAIDs.

4.13   Celebrex Docking

For the docking purpose CB Dock online docking tool is used. It gives us 5 best confirmation results and finest is selected. Celebrex is used as ligand and cox-2, m PGES-2, HNE and tyrosinase are selected as receptors. As the mechanism of action shows that Celebrex inhibits Cox-2 and other enzymes which involve in inflammation. So docking help us to find out the inhibition value, therefore the values were shown in Table 4.40.

TABLE 4.40: Celebrex Docking Scores Via Cb Dock

S.No	Compound	Celebrex
1	Binding Score	-10.4
2	HBD	1
3	HBA	4
4	logP	3.51392
5	Molecular Weight g/mol	381.379
6	Rotatable Bonds	3
7	Grid Map	58
8	Min-energy Kcl/mol	0
9	Max-energy Kcl/mol	1.60E+00
10	Cavity Size	3377

4.14 Celebrex and Anti-Inflammatory Agent Comparison

The comparison between Celebrex and Sapogenin help us to identify the better treatment for infectious diseases. Comparison is being performed through parameters like; ADMET properties and physiochemical properties of celebrex and sapogenins. Celebrex and Sapogenin Lipinski rule of fives were given in Table 4.41.

TABLE 4.41: Celebrex and Sapogenins Lipinski Rule of Fives

S.No	Drug	logP Value	Molecular Weight	H-Bond Acceptor	H-Bond Donor
1	Celebrex	3.51392	381.379 g/mol	4	1
2	Sapogenin	4.6027	490.725 g/mol	5	4

So it is determine that Sapogenin bioactive compound which shows us better result over celebrex (generic drug) according to comparison.

4.14.1 ADMET Properties Comparison

ADMET properties include the values regarding to drug absorption, distribution, metabolism, excretion and toxicity. These values help us to determine the drug activity and efficiency.

4.14.1.1 Toxicity Comparison

The toxicity ranges of Celebrex and Sapogenin were given in Table 4.42. The max tolerated dose for Celebrex is 0.178 and for Sapogenin is -0.888 and oral acute toxicity rat of Sapogenin is greater and chronic toxicity rat of Celebrex is higher.

TABLE 4.42: Toxicity Values of Celebrex & Sapogenin Via PkCSM Tool

S.No	Model Name	Predicted Values	
		Celebrex	Sapogenin
1	Max.tolerated dose(human)	0.178 mg/Kg	-0.888 mg/Kg
2	hERG I inhibitor	No	No
3	hERG II inhibitor	No	No
4	Oral rat acute toxicity	1.975 mol/Kg	2.395 mol/Kg
5	Oral rat chronic toxicity	1.526 mg/Kg	1.462 mg/Kg
6	Hepatoxicity	Yes	No
7	Skin sensitization	No	No
8	t.pyriformis toxicity	0.43 log ug/L	0.304 log ug/L
9	Minnow toxicity	0.86 log mM	1.486 log mM

4.14.1.2 Absorption Properties Comparison

The absorption properties of Celebrex drug and Sapogenin were given in Table 4.43.

TABLE 4.43: Absorption Properties of Celebrex Drug and Sapogenin

S.No	Ligand	Celebrex	Sapogenin
1	Water solubility	-4.45 mol/L	-5.433 mol/L
2	Caco2 permeability	0.839 cm/S	1.055 cm/S
3	Intestinal absorption (human)	92.995 %	95.08 %
4	Skin Permeability	-2.692 log Kp	-3.702 log Kp
5	P-glycoprotein substrate	Yes	Yes
6	P-glycoprotein I inhibitor	Yes	Yes
7	P-glycoprotein II inhibitor	Yes	No

So it is determine that Sapogenin is easily soluble in water as compared to Celebrex and it is permeable to skin. The intestine absorption of Sapogenin is greater as compared to Celebrex.

4.14.1.3   Distribution Properties Comparison

The distribution properties of Celebrex drug and Sapogenin were given in Table 4.44.

TABLE 4.44: Distribution Properties of Celebrex Drug and Sapogenin

S.No	Ligand	Celebrex	Sapogenin
1	VDss (human)	-0.273 L/Kg	0.258 L/Kg
2	Fraction unbound (human)	0.133 Fu	0.151 Fu
3	BBB permeability	-0.931 log BB	-0.514 log BB
4	CNS permeability	-2.052 log PS	-2.995 log PS

4.14.1.4   Metabolic Properties Comparison

The metabolic properties of Celebrex drug and Sapogenin were given in Table 4.45.

TABLE 4.45: Metabolic Properties of Celebrex Drug and Sapogenin

S.No	Ligand	Celebrex	Sapogenin
1	CYP2D6 substrate	No	No
2	CYP3A4 substrate	Yes	Yes
3	CYP1A2 inhibitor	No	Yes
4	CYP2C19 inhibitor	Yes	No
5	CYP2C9 inhibitor	Yes	No
6	CYP2D6 inhibitor	No	No
7	CYP3A4 inhibitor	Yes	No

The CYP3A4 substrate present in both Celebrex and Sapogenin but CYP1A2 inhibitor present only in Sapogenin that helps in the metabolism of drug.

4.14.1.5 Excretion Properties Comparison

The excretion properties of Celebrex drug and Sapogenin were given in Table 4.46.

TABLE 4.46: Excretion Properties of Celebrex Drug and Sapogenin

S.No	Ligand	Celebrex	Sapogenin
1	Total Clearance	0.435 ml/Kg	1.218 ml/Kg
2	Renal OCT2 substrate	No	No

The total clearance value of Sapogenin in the body is greater that helps in the excretion of drug from the body.

4.14.2 Physiochemical Properties Comparison

The comparison between physiochemical properties of Celebrex and Sapogenin is important steps that help us to find out the drug activity manner and biochemical reactivity portion. So the comparison between physiochemical properties of Celebrex and Sapogenin were shown in Table 4.47.

So from physiochemical properties it is determine that the logp value, hydrogen bond acceptor, hydrogen bond donor and molecular weight of Sapogenin are greater as compere to Celebrex. So it means that Sapogenin can act as inhibitor for inhibition of those enzymes which involved in inflammation.

TABLE 4.47: Comparison Between Physiochemical Properties of Celebrex and Sapogenin

S.No	Drugs	logP Value	Rotatable Bonds	H-bond Acceptor	H-bond Donor	Molecular Formula	Molecular Weight
1	Celebrex	3.51392	3	4	1	C17H14F3N3O2S	381.379 g/mol
2	Sapogenin	4.6027	2	5	4	C30H50O5	490.725 g/mol

Chapter 5

Conclusions and Future Prospects

The aim of this research is to identify a compound using computational method for the treatment of infectious diseases that could be used in near future as an efficient drug. After performing data mining studies on literature databases ten ligands were selected for the current research work. The proteins used for virtual screening were COX-2, Human Neutrophil Elastase (HNE), microsomal PGES-2 and Tyrosinase proteins. CB Dock automated version of Auto Dock vina is used for the docking studies. Protein ligand interactions of these ligands were analyzed using Ligplot plus version v.1.4.5. After the detailed analysis of their binding score, physiochemical properties and ADMET properties, Sapogenin is identified as a potent inhibitor for inflammation. From the above mentioned physiochemical and ADMET values it is concluded that the Sapogenin activity in comparison to Celebrex is better in activity. In drug designing, structure based drug designing and lead discovery have been used efficiently. All the software's and tools used in the current research study are reliable and authenticate.

5.1   Future Prospects

Sapogenin follows the Lipinski rule of 5s. So from the current research work we identified that near in future Sapogenin can act as an efficient and a possible

alternative drug for the treatment of Infectious Diseases, which having less side effect as compare to synthetic drugs.

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