CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Screening of active constituents of *Sambucus nigra* against neuraminidase of Influenza Virus A-An *Insilco* study

by

Sumaira Emmanuel

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

in the

Faculty of Health and Life Sciences
Department of Bioinformatics and Biosciences

2024

I dedicate this thesis to my loving and supportive family and friends who have fully helped me in achieving my life goals.

CERTIFICATE OF APPROVAL

Screening of active constituents of *Sambucus nigra* against neuraminidase of Influenza Virus A-An *Insilco* study

by

Sumaira Emmanuel

(MBS223008)

THESIS EXAMINING COMMITTEE

S. No.	Examiner	Name	Organization
(a)	External Examiner	Dr. Sara Mumtaz	NUMS, Rawalpindi
(b)	Internal Examiner	Dr. Muhammad Asad Anwar	CUST, Islamabad
(c)	Supervisor	Dr. Erum Dilshad	CUST, Islamabad

Dr. Erum Dilshad
Thesis Supervisor
August, 2024

Dr. Syeda Marriam Bakhtiar
Head
Dept. of Bioinfo. and Biosciences
August, 2024

Dr. Sahar Fazal
Dean
Faculty of Health and Life Sciences
August, 2024

Author's Declaration

I, **Your Name** hereby state that my MS thesis titled "**Screening of active constituents of *Sambucus nigra* against neuraminidase of Influenza Virus A-An *Insilco* study**" is my own work and has not been submitted previously by me for taking any degree from Capital University of Science and Technology, Islamabad or anywhere else in the country/abroad.

At any time if my statement is found to be incorrect even after my graduation, the University has the right to withdraw my MS Degree.

(Sumaira Emmanuel)
Registration No: MBS223008

Plagiarism Undertaking

I solemnly declare that research work presented in this thesis titled "**Screening of active constituents of *Sambucus nigra* against neuraminidase of Influenza Virus A-An *Insilco* study**" is solely my research work with no significant contribution from any other person. Small contribution/help wherever taken has been duly acknowledged and that complete thesis has been written by me.

I understand the zero tolerance policy of the HEC and Capital University of Science and Technology towards plagiarism. Therefore, I as an author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of any formal plagiarism in the above titled thesis even after award of MS Degree, the University reserves the right to withdraw/revoke my MS degree and that HEC and the University have the right to publish my name on the HEC/University website on which names of students are placed who submitted plagiarized work.

(Sumaira Emmanuel)
Registration No: MBS223008

Acknowledgement

All praise and thanks to the Supreme God to whom we only bow down. I would also like to express my gratitude to my family and friends for their continuous mental and physical support and prayers. I would also wholeheartedly say a big thank you to my supervisor Dr. Erum Dilshad (Associate Professor, Department of Bioinformatics and Biosciences, CUST) for her support with that I would say special thanks to Mr. Maaz (PhD Scholar) for giving his precious time to assist with computational approaches.

Thanks to all.

(Sumaira Emmanuel)

Abstract

Influenza viruses come in four different varieties: A, B, C, and D. Human seasonal flu epidemics are mostly caused by influenza A and B viruses and usually happen in the winter. Influenza A viruses are unique in their ability to cause flu pandemics (global epidemics of flu disease). When a new and different influenza pandemic breaks out, Emergence, human infection, efficient dissemination, and minimal or nonexistent community immunity are all symptoms of a virus. Not known to create human epidemics, influenza C virus infections usually result in moderate sickness. It is unknown if influenza D viruses may infect humans or cause disease, however they are known to mostly afflict cattle and occasionally spread to other species. For this purpose, many plants were exploited to find natural com pounds to work against this virus. The detailed study of the Influenza virus A shows that neuraminidase is target protein which is responsible for division, growth and development of virus. This active compound in Sambucus nigra was studied to be docked against the protein neuraminidase. Ten ligands from different classes were selected for this purpose. These ligands were then screened out based on lipinski rule and through studying the ADMET properties of the ligands. After the docking of the selected ligands with the receptor protein through the CB dock, the lead compound quercetin was selected against the standard drug zanamivir. The docking results of both compounds were visualized via PyMol and were analyzed by the use of LigPlot. The result shows that kaempferol and quercetin can be more effective against neuraminidase rather than zanamivir. However further research has to be carried for investigating kaempferol and quercetin potential in medicinal use.

Contents

Author's Declaration iv

Plagiarism Undertaking v

Acknowledgement vi

Abstract vii

List of Figures xi

List of Tables xii

Abbreviations xiv

1 Introduction 1
- 1.1 Problem Statement . . . 3
- 1.2 Aim and Objectives . . . 3

2 Literature Review 4
- 2.1 Influenza Virus . . . 4
- 2.2 Origin . . . 5
- 2.3 Entry and Life Cycle . . . 7
- 2.4 Symptoms . . . 8
- 2.5 Treatment for Influenza Virus A . . . 8
- 2.6 Oseltamivir and Zanamivir . . . 9
- 2.7 Medicinal Plants . . . 10
- 2.8 *Sambucus Nigra* . . . 10
- 2.9 Taxonomic Hierarchy . . . 11
- 2.10 Molecular Docking . . . 11
- 2.11 Neuraminidase . . . 12
- 2.12 Natural Compounds as Inhibitors of Neuraminidase . . . 13
- 2.13 Inhibitors against Neuraminidase of Influenza Virus A in *Sambucus Nigra* . . . 13

3 Materials And Methods 14
- 3.1 Selection of Disease . . . 15

3.2 Selection of Protein . . . 15
3.3 Determination of Physiochemical Properties of Proteins . . . 15
3.4 Cleaning of Downloaded Protein . . . 16
3.5 Determination of Functional Domains of Target Proteins . . . 16
3.6 Selection of Active Metabolic Ligands . . . 16
3.7 Ligands Preparations . . . 16
3.8 Molecular Docking . . . 17
3.9 Visualization of Docking Result via PyMol . . . 17
3.10 Analysis of Docked Complex via LigPlot . . . 18
3.11 Ligand ADME Properties . . . 18
3.12 Lead Compound Identification . . . 18
3.13 Comparison with Standard Drug . . . 19

4 Results and Discussion 20
4.1 Retrieval of Protein Structure . . . 20
4.1.1 3D Structure of the Protein . . . 20
4.1.2 Physical Properties of Protein . . . 21
4.1.3 Identification of Functional Domains of Protein . . . 22
4.1.4 Structure of Protein Refined for Docking . . . 23
4.2 Ligand Selection . . . 23
4.3 Virtual Screening and Toxicity Prediction through Lipinski Rule of Five . . . 25
4.3.1 Toxicity Prediction . . . 26
4.3.1.1 Kaempferol and Quercetin . . . 27
4.3.1.2 Oleanolic acid and Ursolic Acid . . . 27
4.3.1.3 Ascorbic Acid and Benzaldehyde . . . 28
4.3.1.4 4-Vinylguaiacol and Phenylacetaldehyde . . . 29
4.3.2 Cyanidin-3-glucoside, and Ethyllinoleate . . . 29
4.4 Molecular Docking . . . 30
4.5 Interaction of Ligands and the Target Protein . . . 32
4.6 ADME Properties of Ligands . . . 40
4.6.1 Pharmacodynamics . . . 41
4.6.2 Pharmacokinetics . . . 41
4.6.3 Absorption . . . 41
4.6.4 Distribution . . . 43
4.6.5 Metabolism . . . 45
4.6.6 Excretion . . . 46
4.7 Lead Compound Identification . . . 47
4.8 Drug Identification Against Influenza Virus . . . 48
4.8.1 Zanamivir . . . 48
4.9 Drug ADMET Properties . . . 48
4.9.1 Toxicity prediction of Reference Drug . . . 49
4.9.2 Absorption Properties . . . 49
4.9.3 Distribution Properties . . . 50
4.9.4 Metabolic Properties . . . 50

4.9.5 Excretion Properties 51
4.10 Zanamivir Mechanism of Action 51
4.11 Zanamivir Effects on Body 52
4.12 Zanamivir Docking 53
4.13 Zanamivir Comparison with Lead Compound 53
4.14 ADMET properties Comparison 54
4.14.1 Toxicity Comparison 54
4.14.2 Absorption Properties Comparison 55
4.14.3 Metabolic Properties Comparison 56
4.14.4 Distribution Properties Comparison 56
4.14.5 Excretion Properties Comparison 57
4.15 Physiochemical Properties Comparison 58
4.16 Docking Score Comparison 58
4.17 Docking Analysis Comparison 59

5 Conclusion and Future Prospects **63**
5.1 Recommendations 63

Bibliography **65**

List of Figures

2.1 Structure of Influenza virus [8] 5
2.2 The origin of influenza Virus [13] 6
2.3 Entering and life cycle of the Influenza Virus in human cell 8
2.4 *Sambucus nigra* [19] 11

3.1 Overview of Methodology 14

4.1 Neuraminidase 21
4.2 Functional domains of targeted protein 23
4.3 Neuraminidase cleaned protein 23
4.4 Interaction of kaempferol with the receptor protein 33
4.5 Interaction of quercetin with receptor protein 33
4.6 Interaction of oleanolic acid with receptor protein 34
4.7 Interaction of ursolic acid with receptor protein 34
4.8 Interaction of ascorbic acid with receptor protein 35
4.9 Interaction of benzaldehyde with receptor protein 35
4.10 Interaction of 4vinylgluaiacol with receptor protein 36
4.11 Interaction of phenylacetaldehyde with receptor protein 36
4.12 Interaction of cyanidin-3-glucoside with receptor protein 37
4.13 Interaction of ethyllinoleate with receptor protein 37
4.14 Mechanism action of zanamivir [63] 52
4.15 Interaction of Zanamivir with the receptor 59
4.16 Interaction of Kaempferol with receptor 60
4.17 Interaction of Quercetin with receptor 60

List of Tables

2.1 Taxonomic hierarchy of *Sambucus nigra* 11

4.1 Physical Properties of Neuraminidase 22
4.2 Selected ligands with structural information 24
4.3 gives the value of Lipinski Rule for the selected Ligands 25
4.4 Toxicity values of Kaempferol and Quercetin 27
4.5 Toxicity values of oleanolic acid and ursolic acid 28
4.6 Toxicity values of ascorbic acid and benzaldehyde 28
4.7 Toxicity values of 4-vinylguaiacol and phenylacetaldehyde 29
4.8 Toxicity values of cyanidin-3-glucoside and ethyllinoleate 29
4.9 Docking result of kaempferol, quercetin, oleanolic acid and ascorbic acid 31
4.10 Docking result of benzaldehyde, 4 - vinylgluaiacol, cyanidin- 3 - glucoside, phenylacetaldehyde and ethyl linoleate 31
4.11 Active ligand showing hydrogen and hydrophobic interactions . . . 38
4.12 Absorption Properties of kaempferol, quercetin, oleanolic acid, ursolic acid, and ascorbic acid. 42
4.13 Absorption Properties of benzaldehyde, 4vinylgluaiacol, phenyl acetaldehyde, cyanidin3glucoside and ethyl linoleate 43
4.14 Distribution of kaempferol, quercetin, oleanolic acid, ursolic acid and ascorbic acid. 44
4.15 Distribution of benzaldehyde, 4-vinylgluaiacol, cyanidin-3-glucoside, ethyllinoleate 44
4.16 Metabolic Properties of kaempferol, quercetin, oleanolic acid, and ascorbic acid. 45
4.17 Metabolic Properties of benzaldehyde ,4-vinylgluaiacol, phenylacetaldehyde, cyanidin-3-glucoside and ethyllinoleate 46
4.18 Excretory properties of kaempferol, quercetin, oleanolic acid, and ascorbic acid 47
4.19 Excretory properties of benzaldehyde, 4-vinylgluaiacol, phenylacetaldehyde, cyanidin-3-glucoside, ethyllinoleate. 47
4.20 Toxicity Properties of Zanamivir 49
4.21 Absorption properties of zanamivir 49
4.22 Distribution Properties of Zanamivir 50
4.23 Metabolic Properties of Zanamivir 50
4.24 Excretion Properties of Zanamivir 51
4.25 Docking results of Zanamivir 53

4.26 Lipinski Rule Comparison 54
4.27 Toxicity Properties Comparison 55
4.28 Absorption Properties Comparison 55
4.29 Metabolic Properties Comparison 56
4.30 Distribution Properties Comparison 57
4.31 Excretion Properties Comparison 58
4.32 Physiochemical Properties Comparison 58
4.33 Docking Score Comparison 59
4.34 Docking Analysis Comparison 61

Abbreviations

BBB	Blood-brain barrier
CNS	Central Nervous System
FDA	Food Drug Authority
MRTD	Maximum rate tolerated dose
NA	Neuraminidase
NCBI	National Center of Biotechnology Information
NP	Nucleocapsid Protein
OCT	Organic cation transporter
PDB	Protein Data Bank
RDRP	RNA dependent RNA polymerase
RNP	Ribonucleoprotein
S. nigra	*Sambucus nigra*
URT	Urinary Respiratory Track

Chapter 1

Introduction

The family of viruses Orthomyxoviridae includes influenza viruses. Segmented single-strand RNA segments with negative sense make up this type of virus's genome. The genera A, B, C, and D belong to this family. In terms of human health, genera A and B are important. Occasionally, the nucleoprotein of influenza A and B viruses encapsulates all eight of their generic regions. Influenza Respiratory pathogen or viruses are responsible for significant morbidity and mortality on a global scale. They have an impact on several cellular functions, including apoptosis, autophagy, protein synthesis, and proliferation. Despite being thought of as an inbuilt cellular defense against invasive infectious pathogens, influenza virus has developed the ability to encode viral proteins that modify host cell apoptosis in a manner that helps effective viral replication and spread. To treat influenza infections, new therapies must be developed with an understanding of how host responses are modulated [1].

As pathogenic agents, influenza A viruses can travel across species boundaries. They continue to be a major global challenge today. Despite the fact that influenza A viruses have been identified in a range of birds and animal species including, people. Even in cases where influenza A viruses manage to infiltrate populations other than waterfowl, they frequently lack the ability to effectively adapt and spread among humans. These viruses have the ability to form new lineages only very seldom [2].

Right now, the two influenza virus kinds that coexist and gravely infect humans are influenza A and influenza B. The influenza viruses are additionally categorized into two distinct lineages: B/Yamagata/16/88-like (B/Yam) and B/Victoria/2/87-like (B/Vic). H3N2 and H1N1 are two subtypes of influenza A viruses can cause mild respiratory infections, which might manifest as fever, sore throat, allergies, cough, fatigue, and headache. URTs are usually the only sites of infection. Lower respiratory tract infections which are linked to bacterial or viral pneumonia and can be lethal, particularly in elderly individuals, are frequently the outcome of serious influenza virus infections [3].

Three major IAV subtypes, H1N1, H3N2, and H1N2, have emerged and are spreading throughout swine herds worldwide at this time. Following the 1918 global health crisis, the 1918- like cH1N1 virus was identified and spread globally among pigs. For several decades, the cH1N1 swine virus persisted in pig populations throughout the world. However, in certain regions, like Europe, it vanished and was replaced by newly emerging swine influenza viruses [4].

As scientists look for novel approaches to combat influenza viruses with medications, they are examining plant-based substances with strong antiviral and anti-inflammatory capabilities. Oseltamivir and zanamivir considered a potential drug target, has exhibited effective binding affinity with certain antiviral and anti-inflammatory compounds. Drug discovery and design using computer assisted techniques has significantly advanced over the last thirty years, leading to development therapeutic drugs [5].

Computational methodologies, such as molecular docking, have been instrumental, offering cost- effective solutions and expediting the identification of potential drug candidates more rapidly than manual methods. Medicinal plants have historically been utilized to combat various viral diseases. Efforts have been directed towards identifying small molecules from these plants that exhibit inhibitory activity against viruses. The similarity between the genome sequences of medicinal plants used to treat influenza virus A indicates the potential of neuraminidase as a target site in influenza A. Therefore, the main protease becomes the target site

for screening active compounds derived from medicinal plants against this virus [6].

1.1 Problem Statement

Influenza virus including influenza A, B, C are traditionally known to produce symptoms of common flue and respiratory disorders with only moderate clinical impact. For this we need to discover and have to identify new compounds with antiviral properties having least of side effects and whose availability is easy around the world to minimize the effect of the virus.
In this study, I will target the main neuraminidase of the virus with the active compounds having antiviral properties present in *Sambucus nigra* for the conduction of extensive computational studies through molecular docking.

1.2 Aim and Objectives

This study's main goal is to predict potential inhibitors against influenza virus A by the use of molecular docking of active compounds of *Sambucus nigra* showing antiviral properties with neuraminidase of influenza virus A to control disease.

The objectives of the study include:

1. To identify the probable inhibitory compounds with antiviral properties, present in *A Sambucus nigra* against main neuraminidase of influenza A.
2. To examine a ligand's interactions with a protein complex by performing molecular docking.
3. To find the best of the interacting molecules that shows inhibitory effects against the virus.

Chapter 2

Literature Review

2.1 Influenza Virus

The illness commonly referred as the flu is caused by the influenza virus. Severe symptoms, including as fever, sore throat, headaches, body aches, and respiratory issues, may result from it. Pneumonia and other severe, frequently fatal consequences can result from influenza (flu), a highly contagious viral infection of the respiratory system. It is generally spread by breathing virus-containing droplets from coughs and sneezes, and it can afflict people of all ages. Most cases of the flu are caused by breathing in these contaminated droplets. The flu is a seasonal illness that typically strikes between April and September. The length and intensity of flu seasons change from year to year. It is predicted that during a year with high influenza activity, influenza may be a factor in over 3,300 deaths in Australia [7].

A segmented single-stranded RNA virus with a negative sense is the influenza virus. A compound of several copies of nucleoprotein and RNA dependent RNA polymerase (RdRP) called ribonucleoprotein (RNP) encapsulates each segment. The RNP complex is necessary for the virus's life cycle of the virus because it controls and facilitates both the transcription and the replication of the viral DNA in infected cells [8, 9] (Figure 2.1).

An influenza virion is the infectious particle that is essentially spherical in shape. The lipid membrane of the host cell serves as the virus's outer layer, allowing it to proliferate inside this virus is enveloped. Glycoproteins, or proteins attached to sugars, such as neuraminidase and hemagglutinin, are known as spikes and capable of penetrating the lipid membrane. For example, these proteins function as markers of the influenza virus strain A/H1N1. Antibodies, which are proteins produced by the body to fight disease, against these spikes might offer defence against infection. The HA and NA are essential for the body's defence against the virus. Tamiflu and relenza, two antiviral medications, target the NA protein [10].

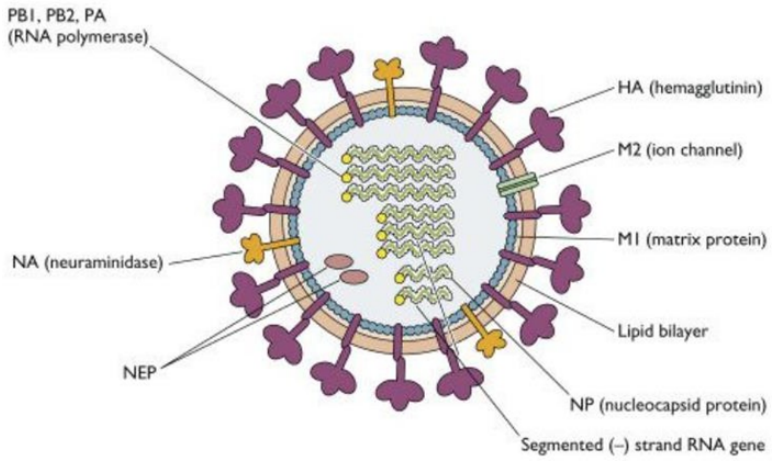


FIGURE 2.1: Structure of Influenza virus [8]

A viral protein called M1, or matrix protein, is located beneath the lipid membrane. It creates a structural shell that gives the membrane strength and stiffness. Eight viral RNAs which codes for one or two proteins, make up the genetic material of influenza virus and are found within the virion, there is a virus as seen in the diagram, each segment is made up of RNA that is connected to the proteins PB1, PB2, PA, and NP. These pieces of RNA carry the influenza virus's genetic material. The virion also includes another protein called NEP [11].

2.2 Origin

One theory is that influenza viruses primarily originate from wild ducks. Reassortment is the process by which human strains of viruses occasionally combine

genetic material from strain of avian virus. Human influenza virus strains that had acquired RNA sequences encoding surface and interior proteins from avian sources were the cause of the epidemic in 1957 and 1968. An antigenic shift occurs when there is a change of subtype of hemagglutinin or the of hemagglutinin and neuraminidase. It been suggested that pigs act as an intermediary in this process since they have been discovered to harbor many interbred and are prone to infection from both avian and human virus strains [12].

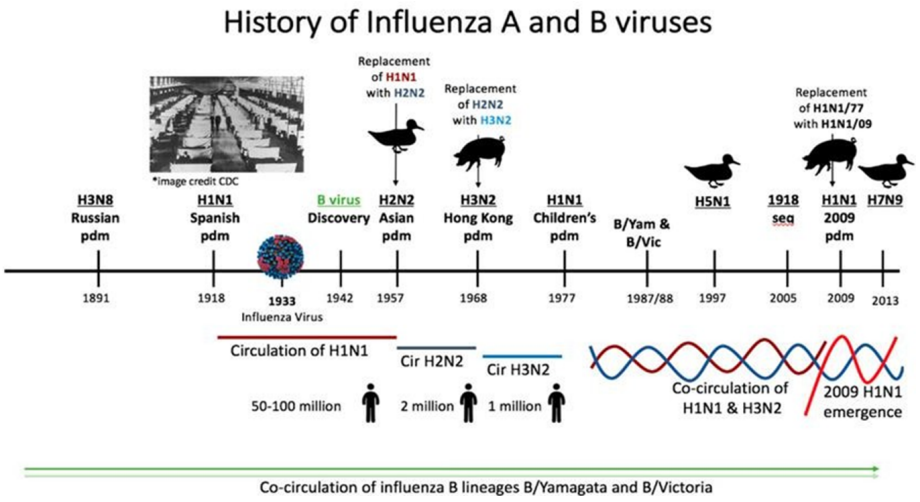


FIGURE 2.2: The origin of influenza Virus [13]

Until recently, there was little evidence that an entirely avian influenza virus might infect humans directly. However, in 1997, the avian H5N1 influenza virus infected 18 people in Hong Kong; six of them died later from linked complications. These viruses were either poorly transmissible or resembled avian influenza viruses, however, the fact that they were separated from sick individuals raises the possibility that humans could contract three different virus strains. During 2003 and 2004, H5N1 epidemic is poultry spread throughout Asia, resulting in at least 32 deaths in Vietnam and Thailand from complication associated to the infection. A highly virulent H7N7 outbreak happened in Dutch poultry farms in 2003. This virus infected 86 poultry handlers and 3 secondary contacts, primarily causing conjunctivitis [13].

2.3 Entry and Life Cycle

The influenza virus goes through multiple stages in its life cycle, including vRNP-mediated entry into the host cell, nucleus penetration, transcription, replication, assembly, and release through budding at the host cell's plasma membrane, as well as vRNP nucleus export and transcription. The lipid membrane of the virus is covered in spikes made of homotrimeric HA proteins.

The host cell's surface membrane contains sialic acid molecules, which the HA spikes attach to. The union of the peptide-containing HA2 and the receptor contacting domain-containing HA1 subunits make up the HA precursor, or HA0. These subunits are joined by disulphide bonds [14]. α (2,3) and α (2,6) are the two primary bonds that sialic acids have with the carbohydrates they bind to in glycoproteins. These are essential for the selectivity with which the HA molecules attach to the sialic acid receptors on the cell surfaces of different species. While viruses from horses and birds can recognize the α (2,3) linkage, human viruses can recognize the α (2,6) linkage. People who eat pork are aware of both. This explains why pigs are a good medium for the fusion of avian and human influenza viruses, which results in the creation of pathogenic viruses [3].

vRNP must enter the nucleus after being released into the cytoplasm for influenza virus transcription and replication, which take place there. The four viral proteins that make up the vRNP are NP, PA, PB1, and PB2. Each of these proteins' NLS has been demonstrated to be able to attach to the cellular nuclear import system and pass into the nucleus [15].

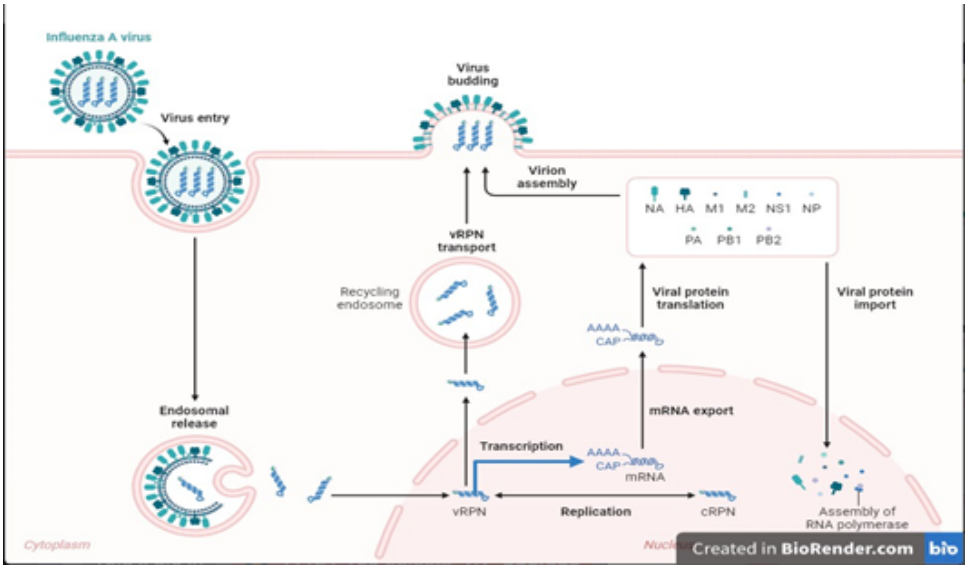


FIGURE 2.3: Entering and life cycle of the Influenza Virus in human cell

2.4 Symptoms

There were no discernible variations in the incidence of diarrhea, vomiting, red throat, or rhinitis cases across all influenza viruses, according to an analysis of the clinical symptoms submitted by patients infected with the virus. An increased number of influenza-positive patients experienced shaking, headaches, aches in the muscles, and joints. A respiratory tract infection manifests as more visible coughing and nasal discharge, which may be followed by ongoing weakness. Death is a possibility; it primarily affects elderly individuals who are already fragile due to a variety of debilitating illnesses, and in most of those cases, a consequence such as pneumonia or bronchitis is the cause.

2.5 Treatment for Influenza Virus A

Research has shown that rimantadine and amantadine are efficacious antiviral treatments for influenza resulting from the type A virus. Unfortunately, viral resistance lessens their efficacy.

Neuraminidase inhibitors, a relatively new class of drugs that included oseltamivir and zanamivir, were first made available in the late 1990s. They successfully block the influenza A and B viruses.

Other than this, the standard treatments for fever management still involve bed rest, fluids, and the use of analgesics. Aspirin is not recommended for use in treating viral infections in children or teenagers with the flu, as it can result in Reye syndrome, a potentially fatal illness [16].

2.6 Oseltamivir and Zanamivir

The antiviral drugs amantadine and rimantadine work well for type A virus-induced influenza cases, but viral resistance may lessen their effectiveness. Neuraminidase inhibitors which were first commercially available in the late 1990s - such as zanamivir and oseltamivir efficiently target influenza A and B viruses. Furthermore, typical interventions such as bed rest, fluid replacement, and analgesic-aided fever management continue to be used. Given the possibility of Reye syndrome, a dangerous illness linked to viral infections, parents of children and teenagers exhibiting flu-like symptoms are advised against giving them aspirin. It has been shown that no antiviral medication suppresses the reproduction of influenza strains resistant to another neuraminidase inhibitor, hence preventing cross-resistance to that agent. Oseltamivir/zanamivir combination therapy reduced viral reproduction at a level comparable to the most effective monotherapy and inhibited influenza viruses that were resistant to both zanamivir and oseltamivir equally well. Conversely, combination therapy was obviously helpful since it prevented the spread of illnesses that were resistant to drugs. These results suggest that preventing the development of resistance can be achieved through combination therapy, which involves the use of two drugs that target a specific viral protein through different binding interactions [17].

2.7 Medicinal Plants

Plants that have demonstrated therapeutic qualities and positive effects on both people and animals are considered medicinal. Medicinal plants have been used for a wide range of ailments since ancient times. Based on their perceptions of smell, taste, and instincts, early humans used a wide range of plants. Some plants were boiled to produce their medicinal compounds, while others were applied topically to heal wounds. For this reason, the therapeutic properties of many different plants have been investigated, and those plants have been a major source of lead treatments [18].

2.8 *Sambucus Nigra*

Black elder, or *Sambucus nigra*, is one of the many medicinal herbs that is traditionally used to treat influenza, the common cold, and conditions similar to the flu. The flu can be effectively treated with elderberry extract. The objective of this research was to examine the antiviral properties of elderberry and its primary active ingredient, cyanidin 3-glucoside (cyn 3-glu). Using flow cytometry analysis, plaque reduction assays, and hemagglutination inhibition assays, the direct effect was investigated. Furthermore, the regulation of pro-inflammatory cytokines was assessed in order to determine the indirect immunomodulatory effect. Early in the influenza virus cycle, elderberry had a modest inhibitory impact; however, in the post-infection phase, this effect became significantly larger. Elderberries, or *Sambucus nigra*, have long been used as an all-natural treatment for illnesses of the upper respiratory tract. Human clinical trials and laboratory investigations have demonstrated the efficacy of elderberry extract in reducing the length and intensity of influenza symptoms caused by various strains of the virus. Regarding the antiviral properties of elderberry and its bioactive constituents, as well as its potential modes of action against the virus such as neutralizing viral glycoproteins a number of theories have been put forth and stimulating the immune system and producing more inflammatory cytokines. These theories are still being investigated [19].

FIGURE 2.4: *Sambucus nigra* [19]

2.9 Taxonomic Hierarchy

Sambucus nigra is the binomial name of the plant belonging to Adoxaceae family. The growing period of the plant is almost 190 to 240 days. It is widely distributed in different regions of the Europe and North America [20]. The taxonomic hierarchy is shown in Table 2.1.

TABLE 2.1: Taxonomic hierarchy of *Sambucus nigra*

S. No	Domain	Eukara
1	Kingdom	Plantae
2	Clade	Tracheophes
3	Clade	Angiosperms
4	Clade	Eudicots
5	Clade	Asteroids
6	Order	Displaces
7	Family	Asteraceaeae
8	Genus	Sambucus
9	Specie	*S. nigra*

2.10 Molecular Docking

For the past thirty years, molecular docking has been used in computer modeling to help with medication creation and to investigate different molecular architectures in biology. Docking is especially helpful for virtual screening chemicals from libraries or databases to examine their functionalities. A major function of docking is to examine the relationship between a ligand and a protein, which is essential

for optimizing lead compounds in drug development. Docking data offer simple classification [21].

Different docking programs estimate possible outcomes of receptor-ligand complexes using one or more search techniques. Because of its adaptability, molecular docking has emerged as a key technique in molecular modeling and drug discovery. An interaction score is produced by docking results, and the precision of this scoring function improves the dependability of ligand pose prediction and, in turn, ligand binding site identification. With this it predicts the binding affiliation which in turn leads to the identification of a potential lead drug in association with the target protein [22].

2.11 Neuraminidase

The enzyme known as neuraminidase is an exosialidase that breaks down the α-ketosidic bond that connects the sialic (N-acetylneuraminic) acid to a nearby sugar residue. The sixth RNA segment codes for the amino acid sequence of NA. For influenza A, nine NA subtypes have been identified; for influenza viruses B and C, only one NA subtype has been identified. The two phylogenic groupings of influenza A NA comprise nine subtypes. The neuraminidases are categorized into two groups, with N2, N3, N6, N7, and N9 belonging to the second group, and subtypes N1, N4, N5, and N8 are in the first group The neuraminidase enzyme of the influenza virus has multiple uses. First, in order to stop viral particles from aggregating, its activity is necessary while freshly generated viral particles are budding off the surface of the infected cell. Furthermore, NA cleaves respiratory tract mucins' neuraminic acid residues, which makes the virus easier to transport to the target cell. The influenza virus NA's polypeptide chain has 470 residues of amino acids Transmembrane domain, head region, cytoplasmic region, and stem region that joins the head to the transmembrane domain, are the domains that make up the three-dimensional structure of Neuraminidase [23].

2.12 Natural Compounds as Inhibitors of Neuraminidase

The main neuraminidase of the influenza virus which controls the replication process is considered an active site for targeting the drugs against the virus. The most common antiviral medications used in clinics to treat influenza are neuraminidase inhibitors. Finding novel NA inhibitors is crucial given the rising incidence of medication resistance. A study conducted in vitro to assess NA inhibition looked at 31 triterpenoids that were extracted from the medicinal mushroom Ganoderma lingzhi. The results showed that the H5N1 and H1N1 neuraminidases were inhibited by two of these triterpenoids, gadoteric acid T-Q and TR [24].

Quercetin (3,3',4',5,7-pentahydroxy-2-phenylchromen-4-one) is the primary component of the flavonoid subgroup called flavanols. Sources of quercetin in nature and their mechanisms of action in various experimental setups that result in antiviral effects. The lack of a successful treatment highlights the need to find novel, potent antiviral drugs. Quercetin targets viral infections at different stages and exhibits possible antiviral action. Proteases, DNA/RNA polymerases, and viral neuraminidase are the primary molecular pathways through which quercetin exerts its antiviral actions. In addition, it affects immunomodulation and changes a number of viral proteins [25].

2.13 Inhibitors against Neuraminidase of Influenza Virus A in *Sambucus Nigra*

There are large number of naturally occurring compounds that can serve as antivirals to inhibit the activity of main neuraminidase of Influenza Virus A. The natural compounds have shown minimal side effects with low toxicity and the important thing is they are easily available to a large mass. The plant *Sambucus nigra* have been used from the earlier times either in the form of medicines, herbs for the curing of cold, pain and flu.

Chapter 3

Materials And Methods

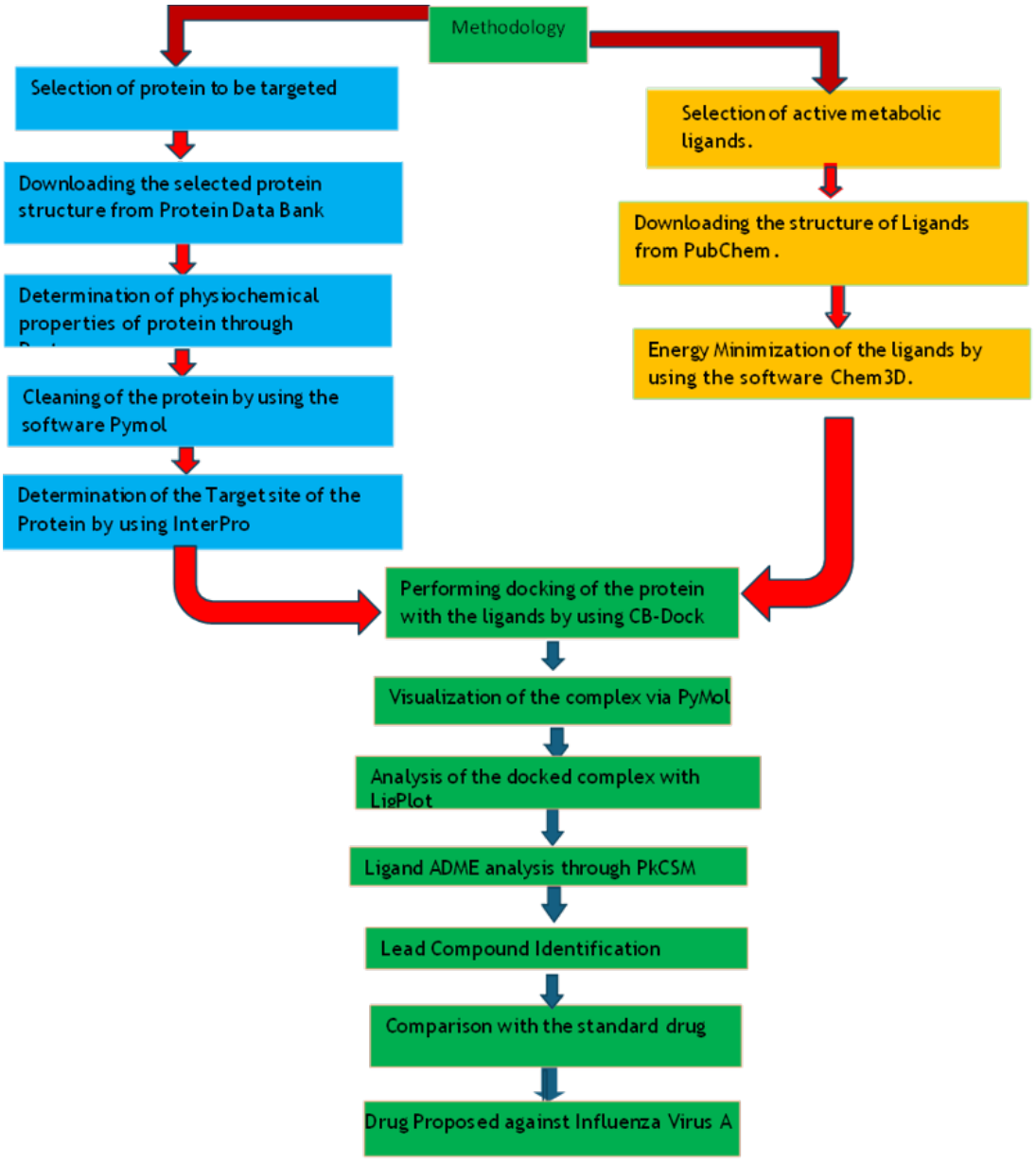


FIGURE 3.1: Overview of Methodology

3.1 Selection of Disease

Due to the current lack of effective medications or vaccinations to treat influenza A-related illnesses, respiratory illnesses that result from influenza A infection, they have become a major concern for communities all over the world. Influenza A is the type of influenza virus that produces the most severe respiratory distress in humans out of influenza B, influenza C, and influenza A. The influenza virus is classified as a single-stranded RNA virus with a negative-sense genome and is a member of the Orthomyxoviridae family. To control the transmission of this virus availability of the drugs have to be ensured. The main neuraminidase of the influenza virus A is identified as to contributes significantly to the virus's ability to replicate. For this purpose, it provides a potential site for drug targeting. Though much work is done but the gaps still remain which needs to be filled.

3.2 Selection of Protein

The particular protein was selected because it is essential to the virus's life cycle. Neuraminidase is necessary for the virus's growth and reproduction. The protein data bank (PDB) provided the crystal structure of the influenza virus neuraminidase, which could be accessed with accession code P03485, DOI doi.org/10.1006 /viro.2000.0727.

3.3 Determination of Physiochemical Properties of Proteins

Understanding the structure and function of a protein requires an understanding of its physical and chemical properties. The number of negatively charged (Asp + Glu) and positively charged (Arg + Lys) residues, as well as molecular weight, isoelectric point, amino acid count, and grand average of hydropathicity, were all analyzed for this using the Expasy tool Protparam.

3.4 Cleaning of Downloaded Protein

After the protein structure was downloaded, other substances are bonded to or connected to the protein needs to be removed which was done by the use of an open-source system Pymol. The linear chain consisting of range 1-306 amino acids was kept referring as the A chain and remaining all the constituents of the protein were eliminated so that further process can be done effectively [26].

3.5 Determination of Functional Domains of Target Proteins

For determining the domains of the target protein, InterPro a database that can analyze a protein was used. It also provided information regarding the families, functional sites and the domains of the protein under study. By inserting the FASTA sequence of the main neuraminidase, the polypeptide binding sites and homodimer interfaces were obtained [27].

3.6 Selection of Active Metabolic Ligands

Those ligands were selected that have previously shown some antiviral and anti-malarial properties. These includes the terpenes, monoterpenes, sesquiterpenes, phenolic compounds, flavonoids, aromatic and quercetin.

3.7 Ligands Preparations

By using the database PubChem, we downloaded the 3-dimensional structure of the above selected ligands. PubChem is under the National Center of Biotechnology Information and is a database that contains the information regarding the chemical molecules. The information stored is related to the chemical names,

molecular formulas, 3-dimensional or simple structures, their isomers, canonical smiles and information regarding the activities of the molecules against the biological assays. The structure of the ligands which were obtained from PubChem were downloaded and then the ligands energy was minimized by using Chem3D pro. At the end pdb format was selected to save the energy minimized structure of the ligand [28].

3.8 Molecular Docking

For performing the molecular docking between the protein and the ligand, the technique used was known as cavity detection guided blind docking, or CB-dock. CB dock finds the sites of docking automatically. CB-Dock is a method of protein and ligand docking which indicates about the sites of bonding, the size and the center is calculated. The box size is adjusted according to the ligand and then docking was performed. The docking is performed through Auto Dock Vina with a better accuracy ratio, CB-dock concentrates on cavity binding [29]. The 3D structures of the ligand and protein were uploaded in sdf and pdb formats, respectively, to start the docking procedure. After this docking is performed. The end result would be 5 different poses of interaction. To select the best pose, we would look upon the lowest possible vina score which is given in KJ/m-1 CB- Dock has provided an interactive 3D visualization of results in 5 different poses. Best pose will be selected on basis of minimum vina score given in (kJ/m1) [30].

3.9 Visualization of Docking Result via PyMol

Over the past few years, the PyMol has emerged as an efficient molecular tool of visualization. The graphics and its ability to view 3D structures have been extraordinary. The docking findings may be more easily accessed and seen with PyMol's plugin, which also improves the display of the data can be easily studied. The pictures of the docking result can be captured also. During all the process the docking results were saved and visualized in the PyMol [31].

3.10 Analysis of Docked Complex via LigPlot

Once we will dock complex with the lowest vina score, the next step was the analysis of the complex. The complex would be in the pdb format. This analysis was done by using the software LigPlot. Protein and ligand interactions are depicted in the schematic diagrams that were automatically created using the supplied pdb file format. Modifications through hydrophobic contacts and hydrogen bonds are involved in these interactions. LigPlot generates a two-dimensional picture of the protein-ligand complex by analyzing hydrophobic and hydrogen bonding interactions [32].

3.11 Ligand ADME Properties

After the analysis the next step was of the study of pharmacokinetic and toxicity properties. The weak candidates of the drug was eliminated by virtual screening. The remaining candidates can be selected as by optimizing neuraminidase through ADME (Absorption, Distribution, Metabolism, and Excretion) investigations, possible treatments for the condition should be developed which is absorption, distribution, metabolism and excretion related to human body was done.

3.12 Lead Compound Identification

After all the work was performed the next step was to find the lead compound. The lead compound was identified after applying the lipinski rule of 5 which includes

1. The log P value of the drug-like compounds must be limited to five.
2. The molecular weight should also be lesser than 500.
3. Hydrogen bond acceptors maximum number should be ten.
4. Hydrogen bond donors' maximum number should be five.
5. The rotatable bonds should be in range of five.

3.13 Comparison with Standard Drug

Oseltamivir and zanamivir which has shown antiviral properties against Influenza variants were selected as a drug standard for comparison against the lead compound. Oseltamivir/zanamivir combination therapy suppressed influenza viruses that were resistant to both zanamivir and oseltamivir just as well, and it decreased viral reproduction at a level comparable to the most successful therapy [33].

Chapter 4

Results and Discussion

4.1 Retrieval of Protein Structure

Neuraminidase was selected as the target protein against the essential components present in Influenza Virus A. It removes terminal sialic acid residues from cellular and viral glycoconjugates via catalysis and enables during virus budding, the terminal sialic acids from the glycosylated HA are cleaved off, releasing the virus. Further aids in the virus's circulation-wide dissemination by progressively eliminating sialic acids from cell surfaces. These cleavages guarantee that the offspring virus spreads effectively from cell to cell and stop it from aggregating.

4.1.1 3D Structure of the Protein

This particular enzyme is referred to be a receptor-destroying enzyme due to its ability to cleave terminal sialic acids off receptors within cells. The mucin of the airway epithelial cells is broken down by cleaving the sialic acid groups on it. It may make it easier for viruses to invade the upper respiratory tract. Most likely contributes to the budding process by joining lipid rafts during intracellular transit. Neuraminidase is a 45.85 kDa protein and has one chain. The PDB contains a large amount of data regarding the protein-ligand complexes. The neuraminidase's

three-dimensional structure was obtained from the protein data bank named 1BJI with the DOI 10.2210/pdb1BJI/pdb.

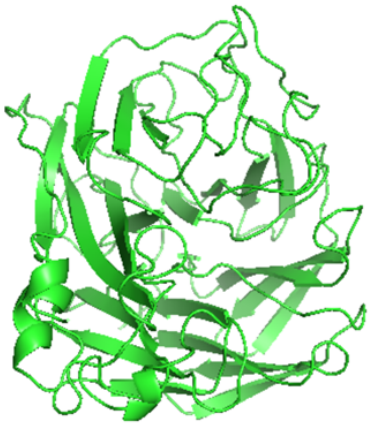


FIGURE 4.1: Neuraminidase

4.1.2 Physical Properties of Protein

For studying the properties of protein neuraminidase, a tool of Expasy named as ProtParam was used. It is an online tool that is used for computing the physical and chemical properties of proteins that are entered in the Swiss-prot or TrEMBL or for the proteins entered by the users. The parameters which are studied include the molecular weight, protein's amino acid composition, atomic composition, theoretical pI, estimated half-life, extinction co-efficient, instability index, aliphatic index, and the last is the grand average of hydropathicity (GRAVY) [34].

With this, the protein showing pI greater than 7 means the basic nature of the protein whereas a pI value lesser than 7 indicates the acidic nature of the protein. Extinction coefficient indicates the light absorption whereas instability index represents stability level of protein if it is lesser than 40 then that means the protein iis stable, any value greater than 40 shows that protein is unstable [35].

The aliphatic index shows the thermostability of a protein. The molecular weight of the protein shows both the positive and the negative amino acid residues. NR indicates the negative residues (Asp+Glu) and PR represents the positive charge residues (Arg + Lys). The low GRAVY value shows the interaction with water

molecules. All the above-mentioned parameters were taken into consideration [35]. The physical properties of the selected protein neuraminidase are discussed in Table 4.1

TABLE 4.1: Physical Properties of Neuraminidase

MW	**pI**	**NR**	**PR**	
52468.85	5.69	47	43	
Ext. Co 1	**Ext. Co 2**	**Instability Index**	**Aliphatic Index**	**GRAVY**
108050	106800	38.42	72.38	-0.437

The above table shows the molecular weight of neuraminidase as 52468.85 which is a collective weight of negative and positive amino acids residues. The pI is 5.69 which indicates that the selected protein is acidic in nature. The values of light absorption in terms of extinction coefficient are 108050 and 106800. The instability index value of 42.37 shows that selected protein neuraminidase is quite a stable protein. Aliphatic index also shows that selected protein is thermostable. Low value of GRAVY shows that neuraminidase has good interactions with water molecules.

4.1.3 Identification of Functional Domains of Protein

For identifying the functional domains InterPro database was used. InterPro helps in finding the functional analysis of proteins and classifies them into families which is done by finding functional domains and other important sites. The active areas of a protein that allow it to interact with other chemicals or proteins are known as its functional domains. The work ID for determining neuraminidase's functional domain is available at www.ebi.ac.uk/interpro.

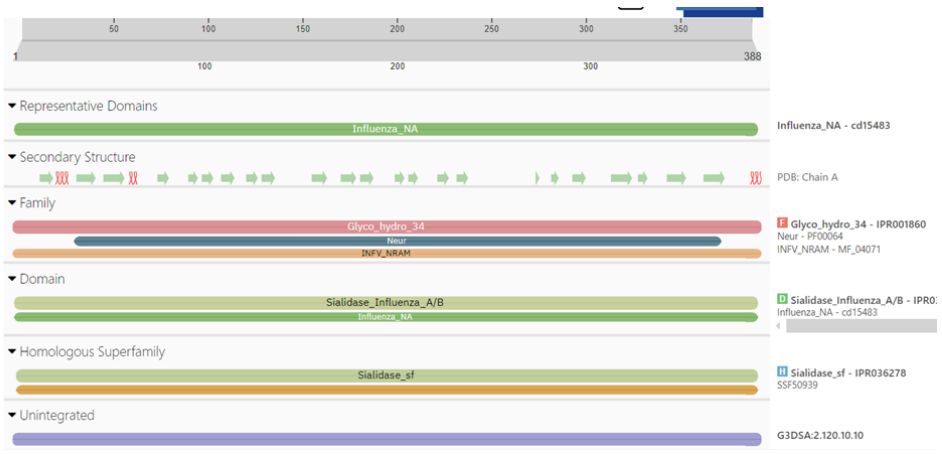


FIGURE 4.2: Functional domains of targeted protein

Figure 4.2 shows the functional domains of the protein to be targeted. It consists of single domain and chain with 2-386 residues.

4.1.4 Structure of Protein Refined for Docking

PyMol was used to improve the protein's structure [36] The water molecules are also removed as shown in figure 4.3, now the protein is ready for docking. It has one domain with 470 amino acids length and 84-468 residues.

FIGURE 4.3: Neuraminidase cleaned protein

4.2 Ligand Selection

The best-resolution structure that was found, the chemical class of the crystal that was linked to the protein, and their binding affinities all had a role in the ligand selection. This process depends on the ligand's conformational selection, which

involves the ligand binding to one conformer more than others in the protein, stabilizing it, and increasing its predominance [37–40].

PubChem, the largest chemical database in the world, was searched to identify the active chemicals from the chosen plant. We downloaded the SDF formatted 3D structures of these chemicals from PubChem. Table 4.2 shows all the selected ligands with the information regarding their structure [37–40]. After downloading the structures of the ligands that were selected, the next step that was performed was minimizing the energy of these ligands. This step is an important one as we can't use simply the downloaded structure as the ligands are unstable and it can directly affect the docking vina scores.

TABLE 4.2: Selected ligands with structural information

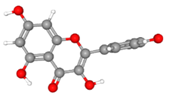
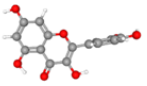
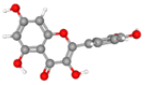
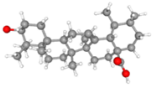
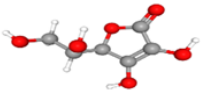
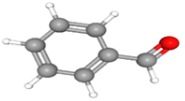
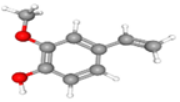
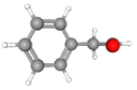
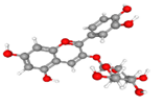
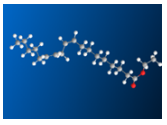
S No.	Ligand Name	Molecular Formula	Molecular Weight	Structure
1	Kaempferol	$C_15H_{10}O_6$	286.24 g/mol	
2	Quercetin	$C_15H_{10}O_7$	302.23 g/mol	
3	Oleanolic acid	$C_{30}H_{48}O_3$	456.7 g/mol	
4	Ursolic acid	$C_{30}H_{48}O_3$	456.7 g/mol	
5	Ascorbic acid	$C_6H_8O_6$	176.12 g/mol	
6	Benzaldehyde	C_7H_6O	106.12 g/mol	

Table 4.2 continued from previous page

S No.	Ligand Name	Molecular Formula	Molecular Weight	Structure
7	4 - Vinylguaiacol	$C_9H_{10}O_2$	150.17 g/mol	
8	Phenyl acetal dehyde	C_8H_8O	120.15 g/mol	
9	Cyanidin-3-Glucoside	$C_{21}H_{21}C_1O$	484.8 g/mol	
10	Ethyllinoleolate	$C_{20}H_{36}O_2$	308.5 g/mol	

4.3 Virtual Screening and Toxicity Prediction through Lipinski Rule of Five

For compounds to be separated as drug-like and non-drug-like lipinski rule of five and ADME properties were followed [41, 42]. Parameters like molecular weight < 500, log P ≤ 5, H-bond donors ≤ 5, and H-bond acceptors ≤ 10 are specified by the Lipinski rule. Orally active substances need to fulfill these requirements. How a drug is administered determines how similar it is to it. If a substance satisfies three of these conditions or more, it is classified as a drug; if it doesn't, it is classified as poorly absorbed [42].

TABLE 4.3: gives the value of Lipinski Rule for the selected Ligands

S. No	**Ligand**	**P-value**	**Weight**	**Acceptor**	**Donor**
1.	Kaempferol	2.284	286.239 g/mol	6	4
2.	Quercetin	1.988	302.238 g/mol	7	5
3.	Oleanolic acid	7.2336	456.711 g/mol	2	2

Table 4.3 continued from previous page

S. No	Ligand	P-value	Weight	Acceptor	Donor
4.	Ursolic acid	7.0895	456.711 g/mol	2	2
5.	Ascorbic acid	-1.4074	176.124 g/mol	6	4
6.	Benzaldehyde	1.4991	106.124 g/mol	1	0
7.	4-Vinylguaiacol	2.0438	150.177 g/mol	2	1
8.	Phenylacetaldehyde	1.428	120.15 g/mol	1	0
9.	Cyanidin-3-Glucoside	-2.614	484.84 g/mol	10	8
10.	Ethyllinoleate	6.363	308.5 g/mol	2	0

The above table shows that out of the 10 ligands, oleanolic acid, ursolic acid, cyanidin-3-glucoside and ethyllinoleate did not follow the lipinski rule of five.

4.3.1 Toxicity Prediction

An online method called PkCSM was used to forecast the ADMET (absorption, distribution, metabolism, excretion, and toxicity) values of medications and bioactive substances. By using this tool, we determined the toxicity of the ligands selected, for these different methods are used to test whether a given ligand is toxic or not [43–45].

With the use of microorganisms, the AMES toxicity test assesses a compound's propensity for mutation. If it shows a positive response, then the ligand is mutagenic which can also act as a carcinogen [43–45]. *T. Pyriformis* toxicity method uses *T. Pyriformis* (protozoa bacteria) toxicity as a toxic endpoint. Any value >-0.5 log ug/L is considered toxic [43–45]. The values predicted in the Minnow toxicity test are used to represent the concentration at which the compound could cause the death of 50% of the minnows. The value below 0.5 mM is regarded as acute toxic [43–45].

The values for MRTD (maximum recommended tolerated dose) give a picture of the starting dose of a certain pharmaceutical, at clinical phase I. Value $\leq$ 0.477 log mg/kg/day is low and a value greater than this value is considered as high [43–45].

For the oral rat chronic test of toxicity, the predicted log value of the lowest observed adverse effect in log mg/kg bw/day is given which relates to the concentration of the compound given that requires the treatment time [43–45].

A hepatotoxicity test predicts that if a compound could affect the liver functioning or not [43–45].

4.3.1.1 Kaempferol and Quercetin

The toxicity values of kaempferol and quercetin are given below. Table 4.4 shows that kaempferol has a high MRTD value. All other test values are in the safe range, that shows that both are not the cause for AMES toxicity and have a safe tolerated dose. They both are the hERG I and II inhibitors. They both have a safe toxic rate with respect to test on rat and on *T. pyriformis* with that they are not toxic to liver and does not provide any sensitivity to skin [44, 45].

TABLE 4.4: Toxicity values of Kaempferol and Quercetin

S. No	Model Name	Predicted Value of Kaempferol	Predicted Value of Quercetin
1.	AMES Toxicity	No	No
2.	Max. tolerated dose (human)	0.531	0.499
3.	hERG I inhibitor	No	No
4.	hERG II inhibitor	No	No
5.	Oral rat acute toxicity	2.449	2.471
6.	Oral rat chronic toxicity	2.505	2.612
7.	Hepatoxicity	No	No
8.	Skin sensitization	No	No
9.	T. pyriformis toxicity	0.312	0.288
10.	Minnow toxicity	2.885	3.721

4.3.1.2 Oleanolic acid and Ursolic Acid

The toxicity values of oleanolic acid and ursolic acid are given below. Table 4.5 indicates that oleanolic acid has a high MRTD value. Both are not sensitive to

skin. Both also causes hepatoxicity and rest all parameters were in safe range [43, 45].

TABLE 4.5: Toxicity values of oleanolic acid and ursolic acid

S. No	Model Name	Predicted Value of Oleanolic acid	Predicted Value of Ursolic acid
1.	AMES Toxicity	No	No
2.	Max. tolerated dose (human)	0.203	0.199
3.	hERG I inhibitor	No	No
4.	hERG II inhibitor	No	No
5.	Oral rat acute toxicity	2.349	2.346
6.	Oral rat chronic toxicity	2.085	2.054
7.	Hepatoxicity	Yes	Yes
8.	Skin sensitization	No	No
9.	T. pyriformis toxicity	0.285	0.285
10.	Minnow toxicity	-0.823	-0.787

4.3.1.3 Ascorbic Acid and Benzaldehyde

The toxicity values of ascorbic acid and benzaldehyde are given below. The Table 4.6 indicate that ascorbic acid has high MRTD values. Apart from these all the toxicity parameters were in the positive range.

TABLE 4.6: Toxicity values of ascorbic acid and benzaldehyde

S. No	Model Name	Predicted Value of Ascorbic acid	Predicted Value of Benzaldehyde
1.	AMES Toxicity	No	No
2.	Max. tolerated dose (human)	1.598	0.997
3.	hERG I inhibitor	No	No
4.	hERG II inhibitor	No	No
5.	Oral rat acute toxicity	1.063	1.803
6.	Oral rat chronic toxicity	3.186	1.959
7.	Hepatoxicity	No	No
8.	Skin Sensitization	No	No
9.	*T. pyriformis* Toxicity	0.285	-0.285
10.	Minnow Toxicity	4.386	1.724

4.3.1.4 4-Vinylguaiacol and Phenylacetaldehyde

The toxicity values through pkCSM are given below. The table 4.7 indicates 4-vinylgluaiacol has high MRTD values and show positive AMES toxicity. Both ligands are skin sensitive, rest all parameters are in safe range [43, 45].

TABLE 4.7: Toxicity values of 4-vinylguaiacol and phenylacetaldehyde

S. No	Model Name	Predicted Value of 4-Vinylgluaiacol	Predicted Value of Phenylacetaldehyde
1.	AMES Toxicity	Yes	No
2.	Max. tolerated dose (human)	1.067	0.892
3.	hERG I inhibitor	No	No
4.	hERG II inhibitor	No	No
5.	Oral rat acute toxicity	2.076	1.695
6.	Oral rat chronic toxicity	2.019	1.801
7.	Hepatoxicity	No	No
8.	Skin Sensitization	Yes	Yes
9.	T. pyriformis Toxicity	0.071	-0.137
10.	Minnow Toxicity	1.957	1.78

4.3.2 Cyanidin-3-glucoside, and Ethyllinoleate

Here table 4.8 indicates that ethyllinoleate is a skin sensation. Apart from this, these ligands has all other values in the given pkCSM range. Regarding AMES toxicity, MRTD, hERG I and II inhibition, rat toxicity, *T. pyriformis* and minnow toxicity, as well as liver toxicity, both are within the acceptable range [44, 45].

TABLE 4.8: Toxicity values of cyanidin-3-glucoside and ethyllinoleate

S. No	Model Name	Predicted Value of Cyanidin3Glucoside	Predicted Value of Ethyllinoleolate
1.	AMES Toxicity	No	No
2.	Max. tolerated dose (human)	0.562	0.009
3.	hERG I inhibitor	No	No
4.	hERG II inhibitor	Yes	No
5.	Oral rat acute toxicity	2.547	1.644
6.	Oral rat chronic toxicity	4.362	3.025

Table 4.8 continued from previous page

S. No	Model Name	Predicted Value of Cyanidin3Glucoside	Predicted Value of Ethyllinoleolate
7.	Hepatoxicity	No	No
8.	Skin Sensitization	No	Yes
9.	T. pyriformis Toxicity	0.285	1.497
10.	Minnow Toxicity	6.937	-1.765

The toxicity values mentioned in the above tables from 4.4 to 4.8 shows that based on toxicity tests like skin sensation, hERGII inhibitors, AMES toxicity, and minnow toxicity we would screened out ursolic acid, oleanolic acid, phenylacetaldehyde, 4vinylgluaiacol, and ethylinoleate. All other ligands passed the toxicity test, but the final screening was based on the overall ADME properties.

4.4 Molecular Docking

Molecular docking is a method that determines the proper structure of the ligand that binds to the binding sites and estimates the binding strength between a ligand and a receptor protein using the vina score function. For docking, the three-dimensional structures of the protein and ligands were employed. This was accomplished by using an online blind auto-docking program called CB-Dock [46, 47]. CB-Dock computes the cavity sizes and predicts the binding sites of the protein. CB-Dock returns the five optimal poses and receptor models after docking. Next, the vina score and cavity size are used to determine the ideal position [46, 47].

Neuraminidase was used as the receptor protein and the ten chosen ligands for molecular docking. The format of the ligands was SDF, and the protein was PDB. CB-Dock used Open Babel and MGL Tools to verify the input files and convert them into the pdbqt format [48]. The receptor cavities were then anticipated by CB-Dock, which also determined the diameters and centers of the top five cavities. Based on the protein-ligand interaction's greatest affinity score, the optimal conformation was selected from these five [48]. Tables 4.9, 4.10 display the ligands that bind to neuraminidase with the highest binding scores.

Table 4.9 shows the docking result of five selected ligands that are kaempferol, quercetin, oleanolic acid and ascorbic acid. It shows that kaempferol has a binding score of -8.1, with 6 accepting and donating any 4 hydrogens. The log P value of this docked result is 2.284. Quercetin shows the docking score of -8.7 with accepting 8or donating 5 hydrogens, and gives a logP value of 1. 988.oleanolic acid, ursolic acid and ascorbic acid has binding score 8.3, and 9.6 and pH. of these compounds 7.2336,7.089, -1.407.

TABLE 4.9: Docking result of kaempferol, quercetin, oleanolic acid and ascorbic acid

S. No.	Compounds	Kaempferol	Quercetin	Oleanolic acid	Ursolic acid	Ascorbic acid
1.	Binding score	-8.1	-8.7	-8.3	-9.6	-5.3
2.	Cavity size	365	365	365	365	365
3.	HBD	4	5	2	2	4
4.	HBA	6	7	2	2	6
5.	LogP	2.284	1.988	7.2336	7.0895	-1.4074
6.	Molecular weight g/mol	286.239	302.238	456.71	456.71	176.12
7.	Rotatable Bonds	1	1	1	1	2
8.	Grid Map	61	61	61	61	61

The table shows that out of these five ligands cyanidin3glucoside was the one showing the highest binding score of -8.5. Benzaldehyde, 4-vinylgluaiacol, phenylacetaldehyde and ethyl linoleate has -5.5, -5.8, 5.9 and -4.9 binding score.

TABLE 4.10: Docking result of benzaldehyde, 4 - vinylgluaiacol, cyanidin- 3 -glucoside, phenylacetaldehyde and ethyl linoleate

S. No.	Compounds	Benzaldehyde	4 - Vinyl guaiacol	Phenyl acetalde-hyde	Cyanidin-3 - Glucoside	Ethyllinoleate
1.	Binding score	-5.5	-5.8	-5.9	-8.5	-4.9
2.	Cavity size	518	518	518	365	518
3.	HBD	0	1	0	8	0
4.	HBA	1	2	1	10	2
5.	LogP	1.4991	2.0438	1.428	-2.614	6.363

Table 4.10 continued from previous page

S. No.	Compounds	Benzaldehyde	4 - Vinyl-guaiacol	Phenyl acetaldehyde	Cyanidin-3 - Glucoside	Ethyllinoleate
6.	Molecular weight g/mol	106.124	150.177	120.151	484.84	308.50
7.	Rotatable Bonds	1	2	2	4	15
8.	Grid Map	69	69	69	61	69

4.5 Interaction of Ligands and the Target Protein

LigPlot and PyMol were used to examine the docking results. The interactions between the ligands and the receptor protein were predicted using LigPlot. Using the 3D coordinates, the graphical system of LigPlot automatically produced 2D depictions of the interactions.LigPlot and PyMol were used to examine the docking results. The interactions between the ligands and the receptor protein were predicted using LigPlot. Using the 3D coordinates, the graphical system of LigPlot automatically produced 2D depictions of the interactions. The major chain components of the receptor protein and the ligand were shown to have hydrophobic contacts and hydrogen bond interactions in these two-dimensional pictures [48]. The 2D diagrams of the ligand-protein interactions are shown in Figures 4.4, and the hydrogen and hydrophobic interactions are displayed in Table 4.19. In particular, Figure 4.4 shows how kaempferol interacts with the receptor protein neuraminidase. It shows that kaempferol forms three hydrogen bonds and five hydrophobic contacts.

Figure 4.5 shows the interaction of quercetin with receptor protein neuraminidase. It shows that quercetin has formed four hydrophobic interactions and six hydrogen bonding attractions.

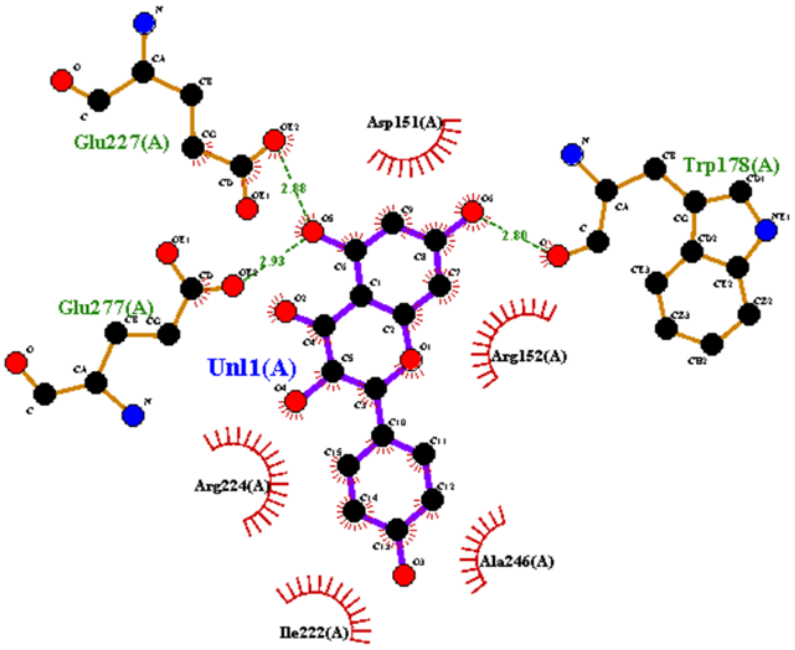


FIGURE 4.4: Interaction of kaempferol with the receptor protein

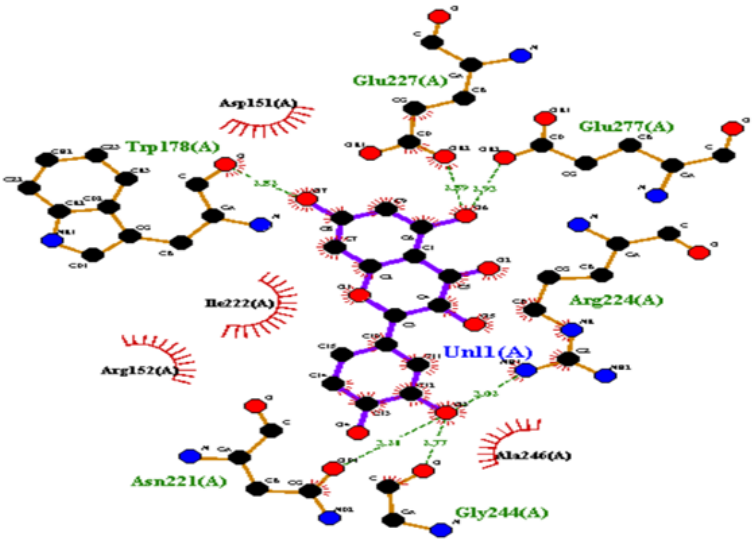


FIGURE 4.5: Interaction of quercetin with receptor protein

Figure 4.6 shows the interaction of oleanolic acid with receptor protein neuraminidase. It shows that oleanolic acid has formed thirteen hydrophobic interactions and one hydrogen bond.

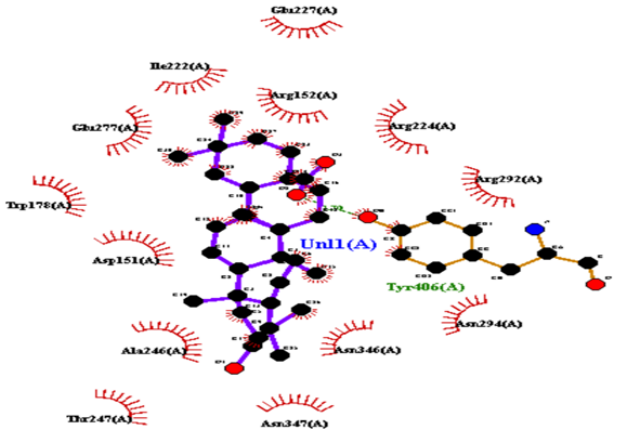


FIGURE 4.6: Interaction of oleanolic acid with receptor protein

Figure 4.7 shows the interaction of ursolic with receptor protein neuraminidase. It shows that ursolic has formed seven hydrophobic interactions and five hydrogen bonds.

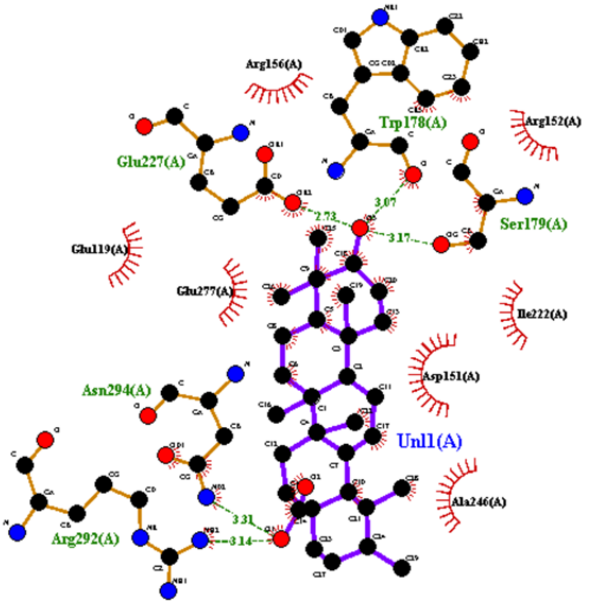


FIGURE 4.7: Interaction of ursolic acid with receptor protein

Figure 4.8 shows the interaction of ascorbic acid with receptor protein neuraminidase. It shows that ascorbic acid has formed four hydrophobic interactions and five hydrogen bonds.

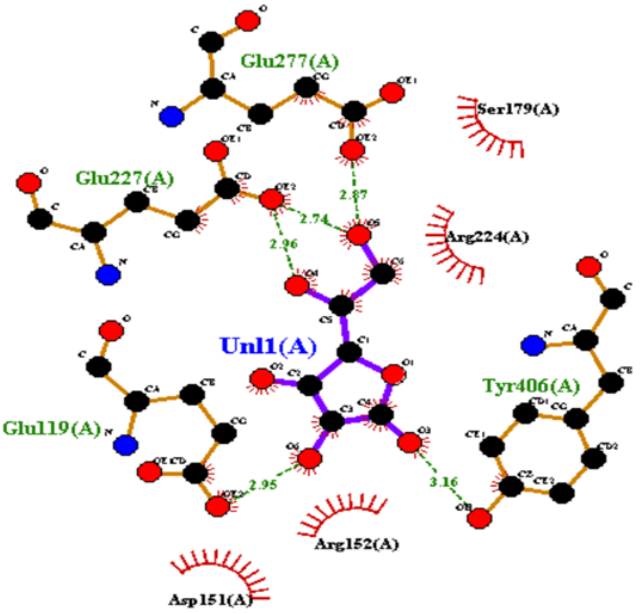


FIGURE 4.8: Interaction of ascorbic acid with receptor protein

Figure 4.9 shows the interaction of benzaldehyde with receptor protein neuraminidase. It shows that there are seven hydrophobic interactions and one hydrogen bond.

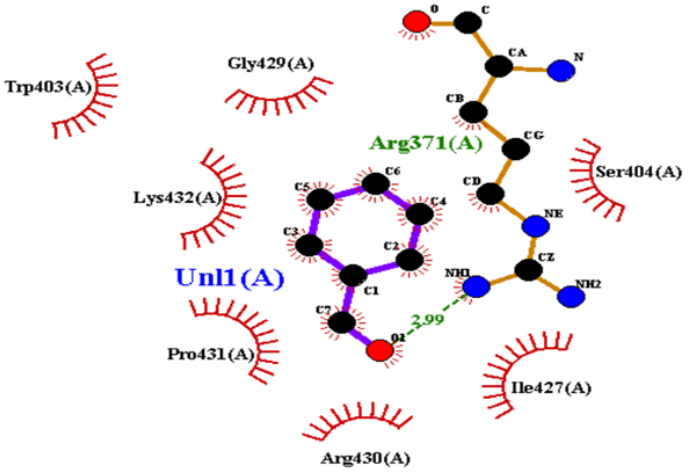


FIGURE 4.9: Interaction of benzaldehyde with receptor protein

Figure 4.10 shows the interaction of 4-vinylgluaiacol with receptor protein neuraminidase. It shows that quercetin has formed seven hydrophobic interactions and no hydrogen bond is formed.

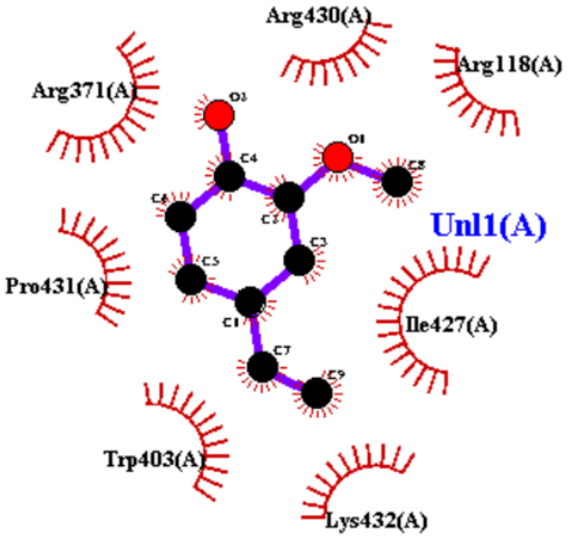


FIGURE 4.10: Interaction of 4vinylgluaiacol with receptor protein

Figure 4.11 shows the interaction of phenylacetaldehyde with receptor protein neuraminidase. It shows that phenylacetaldehyde has formed eight hydrophobic interactions and no hydrogen bond is formed.

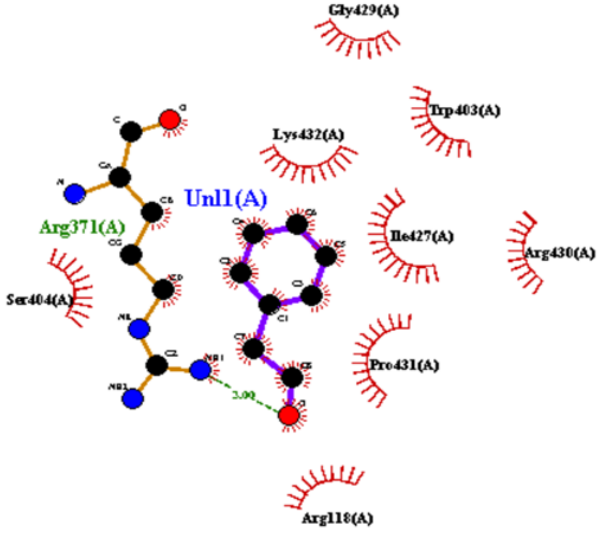


FIGURE 4.11: Interaction of phenylacetaldehyde with receptor protein

Figure 4.12 shows the interaction of cyanidin-3-glucoside with receptor protein neuraminidase. It shows there are six hydrophobic interactions and eight hydrogen bonds.

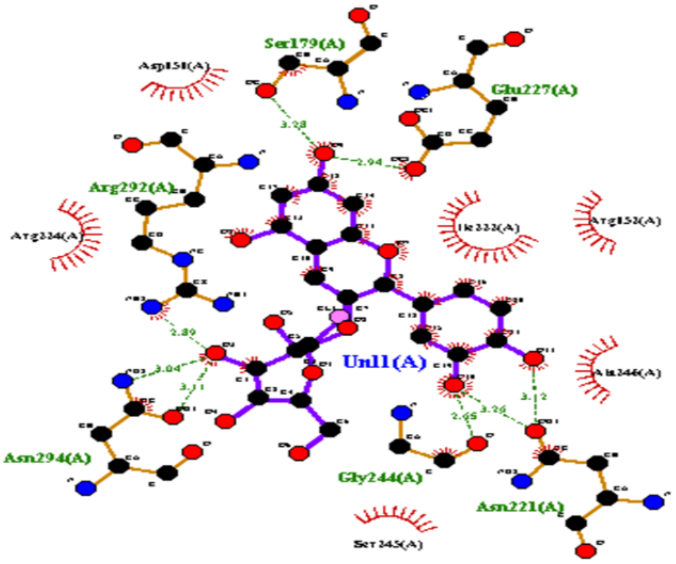


FIGURE 4.12: Interaction of cyanidin-3-glucoside with receptor protein

Figure 4.13 shows the interaction of ethyllinoleate with receptor protein neuraminidase. It shows that has formed thirteen hydrophobic bonds.

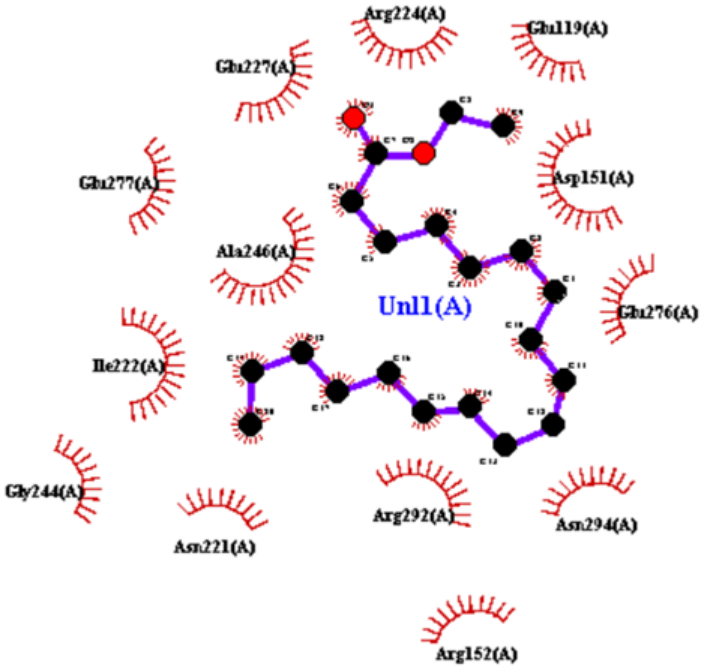


FIGURE 4.13: Interaction of ethyllinoleate with receptor protein

Table 4.11 presents information on the hydrogen and hydrophobic interactions that occur between the receptor protein and the chosen ligands. The values show that oleanolic acid and ethylinoleate forms the highest hydrophobic interactions in

number which is thirteen next is phenylacetaldehyde with 8 hydrophobic bonds, 7 hydrophobic bonds are made by 4-vinylgluaiacol, benzaldehyde and ursolic acid. Whereas kaempferol form 5 and quercetin, ascorbic acid form 4 hydrophobic bonds. The hydrogen bonds formed by cyanidin-3-glucoside are 8 which is the highest in number out of all the selected ligands whereas quercetin forms 6 hydrogen bonds. Ursolic acid and ascorbic forms 5 hydrogen bonds rest kaempferol form 3 and benzaldehyde form 1 hydrogen bond.

TABLE 4.11: Active ligand showing hydrogen and hydrophobic interactions

S.NO	Ligand name	Binding Energy	No. HBs	Bonding Aminoacids	Distance	Hydro-Phobic Bonding
1	Kaempferol	-8.1	3	O6- Trp178-O	2.8	Ala246
				OE2- Glu277- O5	2.93	Arg152
				O5- Glu227- OE2	2.88	Asp151
						Arg224
						Ile222
2	Quercetin	-8.7	6	O-Gly244-O3	2.77	Ala246
				OD1-Asn221-O3	3.21	Arg152
				O3-Arg224-NH1	3.02	Ile222
				OE2-Glu277-O6	2.93	Asp151
				OE2-Glu277-O6	2.89	
				O- Trp178- O7	2.82	
3	Oleanolic acid	-8.3	1	OH- Tyr406- O2	2.7	Asn347
						Asn346
						Asn294
						Arg292
						Arg224
						Arg152
						Glu227
						Ile222
						Glu277
						Trp178
						Asp151
						Ala246
						Thr247

Table 4.11 continued from previous page

S.NO	Ligand name	Binding Energy	No. HBs	Bonding Aminoacids	Distance	Hydro-Phobic Bonding
4	Ursolic acid	-9.6	5	NH2- Arg292- O1	3.14	Ala246
				O1- Asn294- ND2	3.31	Asp151
				O3- Ser179- OG	2.73	Ile222
				O3- Trp178- O	3.07	Arg152
				OE2- Glu227- O3	3.17	Arg156
						Glu119
						Glu277
5	Ascorbic acid	-5.3	5	OE2- Glu119- O6	2.95	Arg152
				OH- Tyr406- O3	3.16	Arg224
				O4- Glu227- OE2	2.96	Ser179
				O5- Glu227 OE2	2.74	Asp151
				O5- Glu227 OE2	2.87	
6	Benzal dehyde	-5.5	1	O1-Arg371-NH1	2.99	Arg430
						Ile427
						Ser404
						Gly429
						Trp403
						Lys432
						Pro431
7	4 - vinyl-gluaiacol	-5.8	-	-	-	Lys432
						Ile427
						Arg118
						Arg430
						Arg371
						Trp403
8	Phenyl acetaldehyde	-5.9	1	NH1-Arg371-O	3	Arg118
						Pro431
						Ile427
						Arg430

Table 4.11 continued from previous page

S.NO	Ligand name	Binding Energy	No. HBs	Bonding Aminoacids	Distance	Hydro-Phobic Bonding
						Lys432
						Trp403
						Gly429
						Ser404
9	Cyanidin-3-glucoside	-8.5	8	OD1-Asn221-O11	2.65	Ser245
				OD1-Asn221-O1O	3.26	Ala246
				O-Gly244-O10	3.12	Ile222
				OD1-Asn294-O3	3.11	Arg152
				ND2-Asn294- O3	3.04	Asp151
				NH2-Arg292-O3	2.89	Arg224
				OG- Ser179- O9	3.28	
				O9- Glu227- OE2	2.94	
10	Ethyllinileate	-4.9	-	-	-	Arg152
						Arg292
						Asn294
						Glu276
						Asp151
						Glu119
						Arg224
						Glu227
						Glu277
						Ala246
						Ile222
						Gly244
						Asn221

4.6 ADME Properties of Ligands

Whether a chemical is natural or synthetic, Lipinski's rule of five is employed as a first step to assess its feasibility as a medication [35]. The ADME attributes are evaluated using the second tool, pkCSM. [48].

4.6.1 Pharmacodynamics

One of the broader terms used in pharmacology is pharmacodynamics It aims to investigate how medications affect the body [38].

4.6.2 Pharmacokinetics

Another word used in pharmacology is pharmacokinetics, which is the study of how medications interact with the body and how they are metabolized once they are ingested. This includes looking into how drugs are absorbed, distributed, metabolized, and excreted [48].

4.6.3 Absorption

The $CaCO_2$ solubility help in predicting the absorption of the drugs which are administered orally. Value >0.90 (log Papp in 10-6 cm/s) is considered as high $CaCO_2$ permeability [45, 46, 48, 49].

The water solubility of the ligands is given as log mol/L. this indicates the compound medicines that are soluble in lipids will be less soluble in water at 25°C than medications that are soluble in water. [48–51].

Intestinal absorption indicates the value or proportion of the compound that will absorb into the intestines. A value less than 30% is considered poorly absorbed [52–55].

P-glycoprotein is an ABC transporter that functions to extrude toxins or other xenobiotics from the cells by acting as a biological barrier [36–39].

P-glycoprotein inhibition can be a therapeutic target or it can act in contradiction [37, 38, 49, 50].

Skin permeability is important for developing transdermal drugs. Any compound with a value

>

-2.5 has a low skin permeability [43–46].

The absorption properties of kaempferol, quercetin, oleanolic acid, ursolic acid, and ascorbic acid are given in Table 4.12.

Table 4.12 shows that kaempferol, quercetin, oleanolic acid, ursolic acid, and ascorbic acid all have low skin permeability with that these kaemperol and quercetin are glycoprotein substrates whereas oleanolic acid, ursolic acid and ascorbic acid are not.

Apart from all these the values of other parameters are in the range.

TABLE 4.12: Absorption Properties of kaempferol, quercetin, oleanolic acid, ursolic acid, and ascorbic acid.

S. No.	Ligands	Kaempferol	Quercetin	Oleanolic acid	Ursolic acid	Ascorbic acid
1.	Water solubility	-3.04	-2.925	-3.074	-3.072	-1.556
2.	CaCO2 permeability	0.032	-0.229	1.17	1.171	-0.255
3.	Intestinal absorption (human)	74.29	77.207	99.931	100	39.154
4.	Skin Permeability	-2.735	-2.735	-2.735	-2.735	-2.955
5.	P-glycoprotein substrate	Yes	Yes	No	No	No
6.	P-glycoprotein I inhibitor	No	No	No	No	No
7.	P-glycoprotein II inhibitor	No	No	No	No	No

Table 4.13 shows the absorption properties of ligands like benzaldehyde, 4 - vinyl gluaiacol, phenyl acetaldehyde, cyanidin-3-glucoside and ethyl linoleate. Table 4.13 reports that all these ligands have low $CaCO_2$ solubility. With that cyanidin-3-glucoside also have low intestinal absorption. Whereas cyanidin-3-glucoside is a glycoprotein substrate. Rest parameters are in safe range.

With that cyanidin-3-glucoside also have low intestinal absorption. Whereas cyanidin-3-glucoside is a glycoprotein substrate. Rest parameters are in safe range.

TABLE 4.13: Absorption Properties of benzaldehyde, 4vinylgluaiacol, phenyl acetaldehyde, cyanidin3glucoside and ethyl linoleate

S. No.	Compounds	Benzal dehyde	4 - Vinyl guaiacol	Phenyl acetalde-hyde	Cyanidin-3 - Glucoside	Ethyl linoleate
1.	Water solubility	-1.198	-1.958	-1.508	-2.904	-7.525
2.	CaCO2 permeabil-ity	1.65	1.499	1.636	0.263	1.608
3.	Intestinal absorp-tion (human)	96.246	91.965	95.899	29.927	92.241
4.	Skin Permeability	-1.667	-2.262	-1.534	-2.735	-2.774
5.	P-glycoprotein sub-strate	No	No	No	Yes	No
6.	P-glycoprotein I in-hibitor	No	No	No	No	No
7.	P-glycoprotein II inhibitor	No	No	No	No	Yes

Based on the information, we get through pkCSM absorption running we can screen several ligands which could be a step behind other ligands. Based on low $CaCO_2$ solubility kaempferol, quercetin, ascorbic acid, and cyanidin-3-glucoside stays aback in the selection of lead compound whereas kaempferol, quercetin and cyanidin-3-glucoside. These are P-glycoprotein substrates rest all are not P-glycoprotein whereas cyanidin-3-glucoside and ascorbic acid has low intestinal absorption.

4.6.4 Distribution

The theoretical volume that indicates the complete dosage of a medication needed to ensure uniform distribution throughout the body at a concentration equivalent to that of blood plasma is represented by the VDss. Whereas a number below 0.71 L/kg implies less distribution in tissues and higher concentration in plasma, a VDss value above 2.81 L/kg indicates greater distribution in tissues than in plasma [44]. Many medications in plasma work with serum proteins to maintain

a balance between their bound and unbound states.

Drugs that bind more strongly to serum proteins have a harder time diffusing across cellular membranes. By preventing external substances from entering brain tissue directly, the blood-brain barrier protects the brain. A substance is deemed effective if its logBB value is more than 0.3 and can easily pass through the blood-brain barrier; a logBB value of less than -1 denotes poor dispersion [45]. The central nervous system (CNS) is penetrated by compounds with a logPS value greater than -2, but not by those with a value less than -3 [46].

The values of the distribution of ligands kaempferol, quercetin, oleanolic acid, ursolic acid and ascorbic acid are given below in Table 4.14. The parameters through which the distribution properties are determined includes VDss which is in the given range to be distributed in the blood and the tissues. The values of the fraction unbound of these ligands shows that out of the total dose this fraction will not be bounded to the protein. All these ligands mentioned in table 4.14 cannot cross the blood brain barriers and the CNS.

TABLE 4.14: Distribution of kaempferol, quercetin, oleanolic acid, ursolic acid and ascorbic acid.

S. No.	Ligands	Kaempferol	Quercetin	Oleanolic acid	Ursolic acid	Ascorbic acid
1.	VDss (human)	1.274	1.559	-1.085	-1.088	0.218
2.	Fraction unbound (human)	0.178	0.206	0	0	0.825
3.	BBB permeability	-0.939	-1.098	-0.14	-0.141	-0.985
4.	CNS permeability	-2.228	-3.065	-1.157	-1.187	-3.217

Table 4.15 shows the distribution properties of benzaldehyde, 4-vinylgluaiacol, cyanidin-3-glucoside, ethyllinoleate. The table indicates all ligands have safe range which is given below.

TABLE 4.15: Distribution of benzaldehyde, 4-vinylgluaiacol, cyanidin-3-glucoside, ethyllinoleate

S. No.	Compounds	Benzal dehyde	4 - Vinyl guaiacol	Phenyl acetal dehyde	Cyanidin-3 - Glucoside	Ethyl linoleate
1.	VDss (human)	0.027	0.118	0.145	1.49	0.306

Table 4.15 continued from previous page

S. No.	Compounds	Benzaldehyde	4 - Vinyl guaiacol	Phenyl acetaldehyde	Cyanidin-3 - Glucoside	Ethyl linoleate
2.	Fraction unbound (human)	0.445	0.322	0.384	0.298	0.015
3.	BBB permeability	0.082	0.289	0.158	-1.374	0.776
4.	CNS permeability	-1.75	-2.042	-1.684	-4.213	-1.562

4.6.5 Metabolism

Cytochrome P450 is an enzyme held responsible for detoxification in the liver. Many drugs get deactivated by this enzyme but certain drugs can be activated. Inhibitors of this enzyme can directly affect the metabolism of drug hence should not be used [51, 56–58]. Similarly, Drug metabolism is mostly dependent on CYP2D6 and CYP3A. Inhibition to these affects the pharmacokinetics of the drug in use [56–59].

The prediction of the metabolism of the ligands is given below.

Table 4.16 shows the metabolic properties of kaempferol, quercetin, oleanolic acid, and ascorbic acid. All the five ligands mentioned are neither the CYP2D6 substrates nor they are CYP2C19, CYP2C9, CYP2D6 and CYP3A4 inhibitors whereas rest parameters are shown in table.

TABLE 4.16: Metabolic Properties of kaempferol, quercetin, oleanolic acid, and ascorbic acid.

S. No.	Ligands	Kaempferol	Quercetin	Oleanolic acid	Ursolic acid	Ascorbic acid
1.	CYP2D6 substrate	No	No	No	No	No
2.	CYP3A4 substrate	No	No	Yes	Yes	No
3.	CYP1A2 inhibitior	Yes	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

Table 4.16 continued from previous page

S. No.	Ligands	Kaempferol	Quercetin	Oleanolic acid	Ursolic acid	Ascorbic acid
7.	CYP3A4 inhibitior	No	No	No	No	No

Table 4.17 shows the Metabolic Properties of benzaldehyde ,4-vinylgluaiacol, phenylacetaldehyde, cyanidin3glucoside and ethyllinioleate. It indicates that all the five ligands mentioned are not CYP2D6, CYP3A4 substrate except ethyllinoleate. Whereas these ligands are not CYP2C19 inhibitior, CYP2C9 inhibitior, CYP2D6 inhibitior and CYP3A4 inhibitior. All ligands are CYP1A2 inhibitior except cyanidin 3 glucoside.

TABLE 4.17: Metabolic Properties of benzaldehyde ,4-vinylgluaiacol, phenylacetaldehyde, cyanidin-3-glucoside and ethyllinoleate

S. No.	Compounds	Benzal dehyde	4 - Vinyl guaiacol	Phenyl acetal dehyde	Cyanidin-3 - Glucoside	Ethyl Linoleate
1.	CYP2D6 substrate	No	No	No	No	No
2.	CYP3A4 substrate	No	No	No	No	Yes
3.	CYP1A2 inhibitior	Yes	Yes	Yes	No	Yes
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

4.6.6 Excretion

The Renal OCT2 substrate acts as a transporter that helps in clearing the drugs and other compounds. Total clearance indicates hepatic clearance which means the drug is metabolized and renal clearance indicates the drug is excreted [52–54, 60]. The excretion values of the ligands are given below.

Table 4.18 shows the excretory properties of kaempferol, quercetin, oleanolic acid, ursolic acid, and ascorbic acid. The table indicates that all these ligands are not

renal OCT2 substrates which means the ligands would not be cleared out of the body and hence the total clearance values are given accordingly.

TABLE 4.18: Excretory properties of kaempferol, quercetin, oleanolic acid, and ascorbic acid

S. No.	Ligands	Kaempferol	Quercetin	Oleanolic acid	Ursolic acid	Ascorbic acid
1.	Total Clearance	0.477	0.407	-0.081	0.083	0.631
2.	Renal OCT2 substrate	No	No	No	No	No

Table 4.19 shows the excretory properties of benzaldehyde, 4-vinylgluaiacol, phenylacetaldehyde, cyanidin-3-glucoside, ethyllinoleate. The table indicates that all these ligands are not renal OCT2 substrates which means the ligands would not be cleared out of the body and hence the total clearance values are given accordingly.

TABLE 4.19: Excretory properties of benzaldehyde, 4-vinylgluaiacol, phenylacetaldehyde, cyanidin-3-glucoside, ethyllinoleate.

S. No.	Compounds	Benzal dehyde	4- Vinyl guaiacol	Phenyl acetal dehyde	Cyanidin-3 - Glucoside	Ethyl linoleate
1.	0.243	0.233	0.331	0.548	2.08	0.631
2.	No	No	No	No	No	No

4.7 Lead Compound Identification

The physiochemical and the pharmacokinetics properties of the ligands determine their fate as drug or non-drug compounds. Lipinski's rule is the first filter and pharmacokinetics is the second filter for this identification. Ursolic acid. oleanolic acid, ethylinoleate and cyanidin-3-glucoside did not follow the lipinski Rule. 3 ligands exceed Log P-value and cyanidin-3- glucoside exceeds hydrogen bond acceptor value.

So, in the first stage, only ursolic acid, oleanolic acid, ethylinoleate and cyanidin-3-glucoside has been knocked out. The next knockout stage was pharmacokinetic

screening. In this screening oleanolic acid and ursolic acid because of being carcinogenic have been knocked out. Phenylacetaldehyde and 4-vinylgluaiacol being skin sensitive has also been knocked out. At the end of this, the compounds left kaemperol and quercetin were selected as the top two compounds as lead.

4.8 Drug Identification Against Influenza Virus

There are a number of medications on the market which are FDA approved that can be used alone or in combination to treat (prophylaxis or therapy) an influenza pandemic. These include ribavirin, interferon, zanamivir and oseltamivir, adamantan (amin)e derivatives (amantadine), and neuraminidase inhibitors [61].

4.8.1 Zanamivir

An antiviral drug called zanamivir is used to treat or prevent influenza A or B virus infections. It is a member of the class of antivirals that are intended to treat viral infections. Zanamivir is an effective treatment for infections brought on by influenza A and B viruses, particularly swine influenza A. However, it is ineffective against non-viral infections, colds, or other flu viruses. If used as soon as possible within two days after the onset of symptoms, it helps lessen flu symptoms such as weakness, headache, fever, cough, congestion, and sore throat by about one to 1.5 days. If you have had close contact with someone who has the flu, you may also want to consider taking zanamivir as a preventive step. It's crucial to understand that zanamivir does not stop the flu from spreading [55].

4.9 Drug ADMET Properties

The program pkCSM, which was previously described, is used to examine the drug's ADMET characteristics.

4.9.1 Toxicity prediction of Reference Drug

Table 4.20 presents the toxicity profile of zanamivir. Since zanamivir's other values fall within positive ranges and its parameters show that it is not hepatotoxic, it is not an inhibitor of hERG I and hERG II, albeit it may cause some skin sensitivity. Also regarded as acceptable is the dosage value of 0.454. Further evidence that zanamivir is not carcinogenic comes from the lack of AMES toxicity.

TABLE 4.20: Toxicity Properties of Zanamivir

S. No	Model Name	Predicted Value
1.	AMES Toxicity	No
2.	Max. tolerated dose (human)	0.454
3.	hERG I inhibitor	No
4.	hERG II inhibitor	No
5.	Oral rat acute toxicity	2.483
6.	Oral rat chronic toxicity	2.726
7.	Hepatoxicity	No
8.	Skin sensitization	No
9.	T. pyriformis toxicity	0.285
10.	Minnow toxicity	5.813

4.9.2 Absorption Properties

The absorption properties of zanamivir are displayed in Table 4.21. Kanamivir has an extremely low solubility in both water and $CaCO_2$, according to the data. Even though it absorbs poorly via the intestinal tract, its levels are still safe. Zanamivir's reduced skin permeability is another feature. It has been determined that it is a P-glycoprotein substrate, however it inhibits P-glycoprotein II but does not inhibit P-glycoprotein I as well.

TABLE 4.21: Absorption properties of zanamivir

S. No.	Reference drug	Zanamivir
1.	Water Solubility	-2.892
2.	$CaCO_2$ Solubility	-0.62
3.	Intestinal Absorption (human)	21.234

Table 4.21 continued from previous page

S. No.	Reference drug	Zanamivir
4.	Skin Permeability	-2.735
5.	P-glycoprotein substrate	Yes
6.	P-glycoprotein I inhibitor	No
7.	P-glycoprotein II inhibitor	No

4.9.3 Distribution Properties

The parameters of zanamivir's distribution are listed in Table 4.22. Given the conditions, it is possible that the medicine does not spread widely throughout the body due to the low VDss value. The blood-brain barrier and the central nervous system (CNS) can both be penetrated by zanamivir.

TABLE 4.22: Distribution Properties of Zanamivir

S. No.	Reference Drug	Zanamivir
1.	VDss (human)	-0.08
2.	Fraction unbound (human)	0.393
3.	BBB Permeability	-1.238
4	CNS Permeability	-4.783

4.9.4 Metabolic Properties

The metabolic characteristics of zanamivir are listed in Table 4.23, which indicates that it is not a substrate for either CYP2D6 or CYP3A4. Furthermore, zanamivir does not inhibit CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4, as indicated by table 4.23.

TABLE 4.23: Metabolic Properties of Zanamivir

S. No	Reference Drug	Zanamivir
1.	CYP2D6 substrate	No
2.	CYP3A4 substrate	No
3.	CYP1A2 inhibitor	No
4.	CYP2C19 inhibitor	No

Table 4.23 continued from previous page

S. No	Reference Drug	Zanamivir
5.	CYP2C9 inhibitor	No
6.	CYP2D6 inhibitor	No
7.	CYP3A4 inhibitor	No

4.9.5 Excretion Properties

The excretion properties of zanamivir are shown in Table 4.24. The details of zanamivir's bodily excretion are given in the table. It suggests that zanamivir does not rely on renal OCT2 as a substrate, implying that this pathway is not necessary for drug clearance. Furthermore, the hepatic and renal clearance processes are taken into account with the total clearance value of -0.347 being provided.

TABLE 4.24: Excretion Properties of Zanamivir

S. No.	Reference Drug	Zanamivir
1.	Total Clearance	0.347
2.	Renal OCT2 Substrate	No

4.10 Zanamivir Mechanism of Action

An antiviral drug called zanamivir is used to treat and prevent influenza A and B. It works by preventing the viral enzyme neuraminidase from doing its job. The reproduction of the influenza virus requires neruminidase (NA). It works by slicing off sialic acid residues from the surface of freshly produced viral particles and host cells. In order to release fresh virions from infected cells and stop virion aggregation, this cleavage is essential. By cleaving sialic acids, NA helps the virus spread through the respiratory tract and facilitates the penetration of mucus in the respiratory tract. Zanamivir is a neuraminidase inhibitor Zanamivir mimics the natural substrate of neuraminidase and binds to the enzyme's active site. This binding action blocks the neuraminidase enzyme from cutting sialic acid residues, thereby preventing its normal function. This inhibition results in newly

formed viral particles remaining attached to the host cell surface or to each other, which prevents the release and spread of the virus. Zanamivir binds to the highly conserved active site of the neuraminidase enzyme. Once bound, it blocks the enzymatic activity of neuraminidase. The lack of neuraminidase activity prevents the cleavage of sialic acid residues. Consequently, progeny virions cannot efficiently detach from the host cell to infect new cells [62].

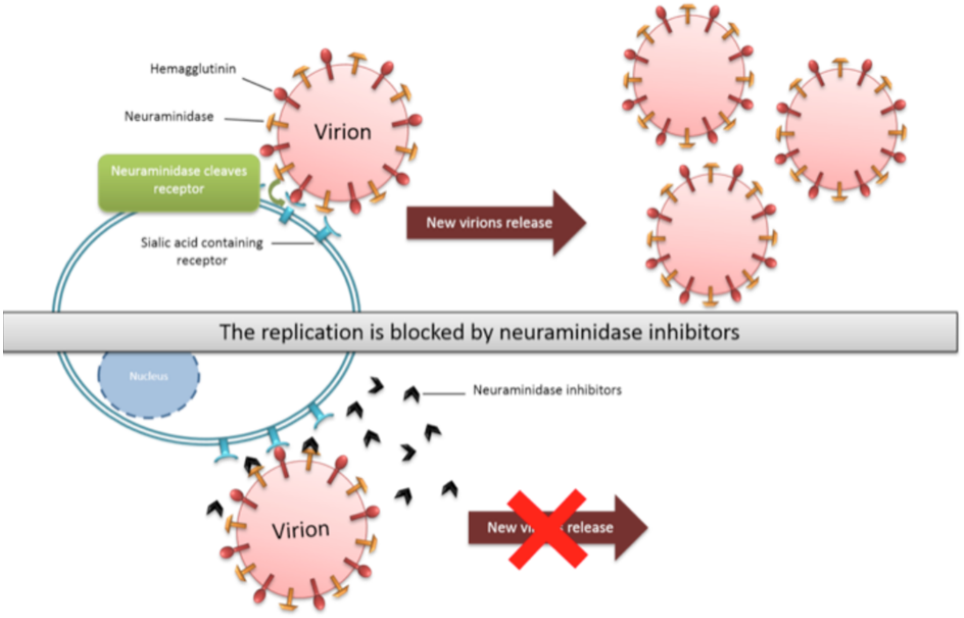


FIGURE 4.14: Mechanism action of zanamivir [63]

4.11 Zanamivir Effects on Body

People with lung conditions including asthma or chronic obstructive pulmonary disease (COPD) may experience wheezing, dyspnea, or dyspnea when using zanami vir. If you have these side effects after taking the drug, stop taking it right once and consult a doctor. Bronchospasm, or wheezing, can occur in patients with asthma or long-term respiratory conditions, so it's best to keep an inhaled bronchodilator on hand for immediate relief. This medicine may also cause serious allergic responses, such as potentially fatal anaphylaxis, which calls for prompt medical attention [64]. If any of the following occur in you or your child: itching, hives, if you have hoarseness, breathing problems, swallowing issues, or hand, cheek,

or mouth swelling, discontinue taking this drug and get medical help right once. Furthermore, especially in kids and teenagers, zanamivir may agitate, irritate, or trigger strange behaviors that could be harmful. A physician or pediatrician should be notified right away if caregivers see any of these adverse effects. In [63]

4.12 Zanamivir Docking

Table 4.25 shows the docking result of zanamivir. The table indicates that zanamivir has a binding score of -7.

TABLE 4.25: Docking results of Zanamivir

Compound	Binding Score	Cavity Size	HBD	HBA	Log P	Molecular Weight	Rotatable Bonds	Grid Map
Zanamivir	-7	518	7	7	-3.7855	332.313	6	69

Strong binding is indicated by the zanamivir and neuraminidase docking studies. One of Lipinski's principles is broken by zanamivir, which forms seven hydrogen bond donors and seven hydrogen bond acceptors. It also has six rotatable bonds in it.

4.13 Zanamivir Comparison with Lead Compound

For the purpose of evaluating bioavailability, efficacy, safety, and drug-likeness, the lead compounds quercetin and kaempferol are compared with the conventional medication zanamivir in terms of their physicochemical and pharmacokinetic properties. Table 4.26 demonstrates how zanamivir violates one of Lipinski's principles for H-bond donors because it possesses seven. While quercetin and kaempferol adhere to all Lipinski rule parameters for LogP, molecular weight, H-bond acceptor, and H-bond donor, the Lipinski rule states that it should be 5.

TABLE 4.26: Lipinski Rule Comparison

S.No.	Name of compound	Log p-value	Molecular weight g/mol	H-bond acceptor	H-bond donor
1	Zanamivir	-3.7855	332.313	7	7
2	Kaempferol	2.284	286.239	6	4
3	Quercetin	1.988	302.238	7	5

4.14 ADMET properties Comparison

In order to determine which drug candidate is better, ADMET attributes are compared to assess the lead compound's and the drug's toxicity, distribution, metabolism, and excretion [56].

4.14.1 Toxicity Comparison

Nine models are used to analyze the toxicity of the lead chemical and the standard medication. According to Model 1 for AMES toxicity, neither the lead chemical nor the conventional medication is mutagenic. Values equal to or less than 0.477 log mg/kg/day are classified as low by Model 2, which determines the maximum tolerable dose; larger values are classified as high. According to the table, there is a low tolerable dose value for quercetin. The third model involves hERG I and II inhibitors, neither of which is an inhibitor. The relative toxicity is evaluated using the fourth model of oral rat acute toxicity. Model 5 of oral rat chronic toxicity provides the lowest dose values that could have a negative outcome. Hepatotoxicity Model 6 indicates that a medicine may harm the liver. The table demonstrates that kaempferol, quercetin, and zanamivir are not hepatotoxic. The number seven is used to verify the dermal goods model's sensitivity to the skin. The lead chemical and the standard are not skin-sensitive. To test for toxicity, Model 8 employs T. Pyriformis, while Model 9 uses minnows. The zanamivir comparative toxicity values are displayed in Table 4.27.

TABLE 4.27: Toxicity Properties Comparison

Sr	Model Name	Zanamivir	Kaempferol	Quercetin
1	AMES Toxicity	No	No	No
2	Max. tolerated dose (human)	0.454	0.531	0.499
3	hERG I inhibitor	No	No	No
4	hERG II inhibitor	No	No	No
5	Oral rat acute toxicity	2.483	2.449	2.471
6	Oral rat chronic toxicity	2.726	2.505	2.612
7	Hepatoxicity	No	No	No
8	Skin sensitization	No	No	No
9	T.pyriformis toxicity	0.285	0.312	0.288
10	Minnow toxicity	5.813	2.885	3.721

4.14.2 Absorption Properties Comparison

Six models serve as the basis for the absorption parameter. The compound's solubility in water at 25 is indicated by the water solubility model. When a medicine is administered orally, its absorption can be anticipated using the $CaCO_2$ solubility model. High intestine absorption is defined as values larger than 0.90, indicating that kaempferol, quercetin is absorbed more than zanamivir. Less than 30% on the intestinal absorption model is regarded as insufficient absorption. The findings for the lead and standard compounds show chrysoplenetin has a high intestine rate of absorption. The two compounds pass the skin penetration test for transdermal medicines, as shown by the skin permeability model, which considers values less than log Kp > -2.5 to be low. P-glycoprotein's substrate model is extremely poorly absorbed. Because P-glycoprotein serves as a biological barrier and an ABC transporter, the P-glycoprotein substrate model is important. Zanamivir, kaempferol and quercetin act as the substrates.

TABLE 4.28: Absorption Properties Comparison

Sr	Reference drug	zanamivir	Kaempferol	Quercetin
1	Water Solubility	-2.892	-3.04	-2.925
2	CaCO2 Solubility	-0.62	0.032	-0.229
3	Intestinal Absorption (human)	21.234	74.29	77.207

Table 4.28 continued from previous page

Sr	Reference drug	zanamivir	Kaempferol	Quercetin
4	Skin Permeability	-2.735	-2.735	-2.735
5	P-glycoprotein substrate	Yes	Yes	Yes
6	P-glycoprotein I inhibitor	No	No	No
7	P-glycoprotein II inhibitor	No	No	No

4.14.3 Metabolic Properties Comparison

As an enzyme involved in detoxification, cytochrome P450 is mostly located in the liver and is responsible for oxidizing foreign substances so that the body may more quickly eliminate them. It can deactivate certain medications or activate others. It is crucial to determine whether a chemical is a P450 substrate or not, as well as whether it inhibits P450. As can be seen in table 4.29, zanamivir lacks a CYP2D6 substrate, CYP3A4 substrate, CYP1A2 inhibitor, CYP2C19 inhibitor, CYP2C9 inhibitor, CYP1A2 inhibitor, and quercetin inhibitor. Kaempferol and quercetin are both CYP1A2 inhibitors.

TABLE 4.29: Metabolic Properties Comparison

S. No.	Reference Drugs	Zanamivir	Kaempferol	Quercetin
1.	CYP2D6 substrate	No	No	No
2.	CYP3A4 substrate	No	No	No
3.	CYP1A2 inhibitior	No	Yes	Yes
4.	CYP2C19 inhibitior	No	No	No
5	CYP2C9 inhibitior	No	No	No
6	CYP2D6 inhibitior	No	No	No
7	CYP3A4 inhibitior	No	No	No

4.14.4 Distribution Properties Comparison

Table 4.30 displays the relative distribution characteristics of quercetin, kaempferol, and zanamivir. Four models form the foundation of the distribution parameter. When the medication's volume of distribution exceeds 2.81 L/kg, it indicates that the drug is more evenly distributed in the tissues than in the blood plasma.

The volume of distribution measures the drug's uniform distribution in the blood plasma. The values of zanamivir, quercetin, and kaempferol are appropriate. The second model is predicated on the proportion of medicines that are unbound in the plasma, as bounded drugs have an impact on drug efficiency. The quantity of medication that is still unrestricted is indicated by the value. In terms of blood-brain barrier permeability, a drug can pass through it more readily if its value is higher than 0.3 log BB; if it is lower than -1 log BB, the drug is either not distributed to the brain or is distributed poorly. Zanamivir would be poorly dispersed to the brain since, as can be seen from these numbers, it has a low value. Comparably, the central nervous system (CNS) model is predicated on the idea that drugs with a logPS value more than -2 can readily enter the CNS, whereas drugs with a logPS value less than -3 cannot enter the CNS. Because of its low value, zanamivir cannot pass through the central nervous system.

TABLE 4.30: Distribution Properties Comparison

S. No.	Reference drug	Zanamivir	Kaempferol	Quercetin
1.	VDss (human)	-0.08	1.274	1.559
2.	Fraction unbound (human)	0.393	0.178	0.206
3.	BBB permeability	-1.238	-0.939	-1.098
4.	CNS permeability	-4.783	-2.228	-3.065

4.14.5 Excretion Properties Comparison

In order to evaluate the medication dosage rates, the total clearance value—which combines hepatic and renal clearance—is crucial. Compared to zanamivir, kaemp ferol and quercetin have higher overall clearance. The renal OCT2 (organic cation transporter 2) model, which is the second model, aids in the renal clearance of medications and other substances. Existing as an OCT2 substrate may have negative consequences when combined with inhibitors. Consequently, quercetin, kaempferol, and zanamivir are not renal OCT2 substrates. The excretory characteristics of quercetin, kaempferol, and zanamivir are displayed in Table 4.31.

TABLE 4.31: Excretion Properties Comparison

S. No.	Reference drug	Zanamivir	Kaempferol	Quercetin
1.	Total Clearance	0.347	0.477	0.407
2.	Renal OCT2 substrate	No	No	No

4.15 Physiochemical Properties Comparison

The physiochemical properties of the compounds are investigated in order to ascertain their basic characteristics. In contrast to kaempferol, which has ten hydrogen atoms, six oxygen atoms, and fifteen carbon atoms 12 carbon, 20 hydrogen, 4 nitrogen, and 7 oxygen atoms make up zanamivir according to this screening. Zanamivir is exempt from the Lipinski rule and is capable of donating seven hydrogen atoms. Quercetin can give four or five hydrogen atoms, indicating the oxidation state, in contrast to kaempferol. Zanamivir, quercetin, and kaempferol can give 7,6. Even while zanamivir's Log P value is lower than that of quercetin and kaempferol, its molecular weight is significantly higher. When rotatable bonds are compared, zanamivir has six, while quercetin and kaempferol only have one. The comparison of the physiochemical characteristics of quercetin, kaempferol, and zanamivir is presented in Table 4.32.

TABLE 4.32: Physiochemical Properties Comparison

S.No	Drug	Mol Formula	H bond donor	H bond acceptor	Log P value	Mol weight	Rotatable Bonds
1	Zanamivir	C12H20N4O7	7	7	-3.7855	332.31	6
2	Kaempferol	C15H10O6	4	6	2.284	286.239	1
3	Quercetin	C15H10O7	5	7	1.988	302.238	1

4.16 Docking Score Comparison

The best binding score was obtained by docking the lead drug and the standard compound against neuraminidase. Table 4.33 indicates that kaempferol and quercetin, the main ingredient, have a significantly higher vina score than

zanamivir, the conventional medication. Zanamivir has a binding score of -7, kaempferol of -8.1, and quercetin of 8.7, all of which are higher than those of the prescribed medication. This indicates that both of them are more effective than zanamivir at binding to or blocking neuraminidase.

TABLE 4.33: Docking Score Comparison

S. No.	Compound	Binding Score
1	Zanamivir	-7
2	Kaempferol	-8.1
3	Quercetin	-8.7

4.17 Docking Analysis Comparison

LigPlot analyzes the docking results based on the quantity of hydrophobic contacts, hydrogen bonds, and interacting amino acids.and that of steric interactions.

Figure 4.15 and 4.16 shows the docking results of zanamivir, kaempferol and quercetin. Figure 4.15 shows that zanamivir has formed eight hydrogen bond and seven hydrophobic interactions.

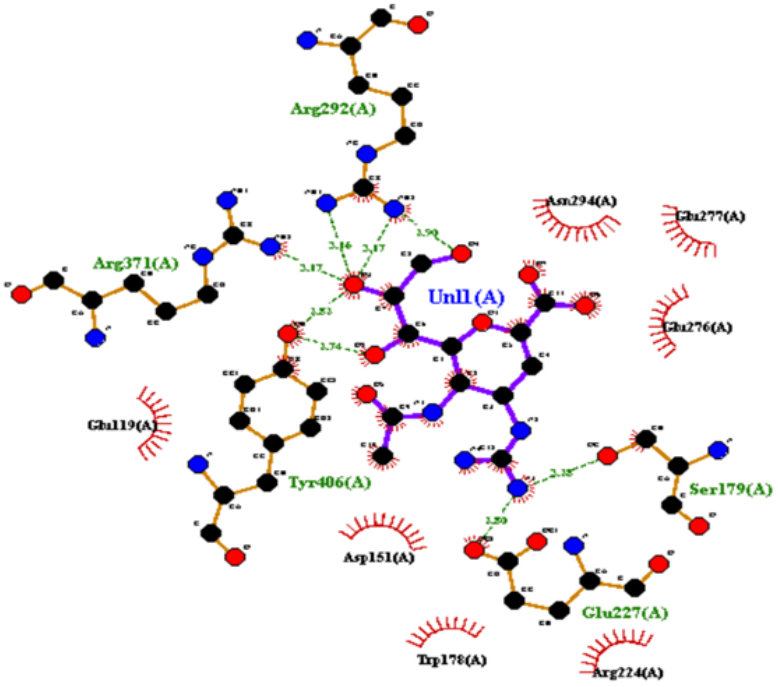


FIGURE 4.15: Interaction of Zanamivir with the receptor

Figure 4.16 shows that kaempferol has formed three hydrogen bonds and five hydrophobic interactions.

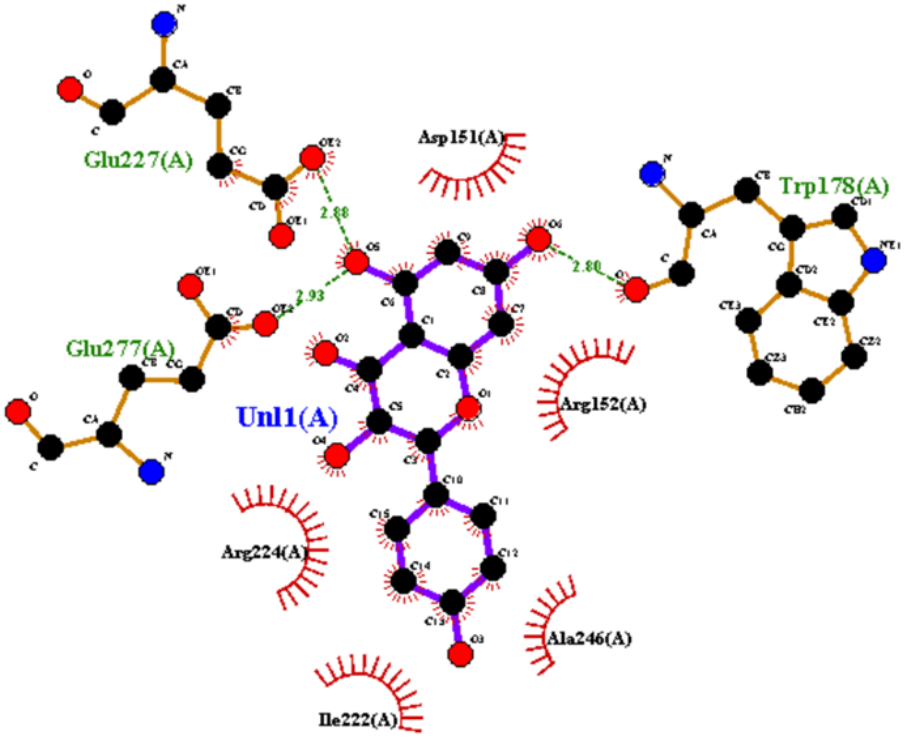


FIGURE 4.16: Interaction of Kaempferol with receptor

Figure 4.17 shows that quercetin has formed six hydrogen bonds and four hydrophobic interactions.

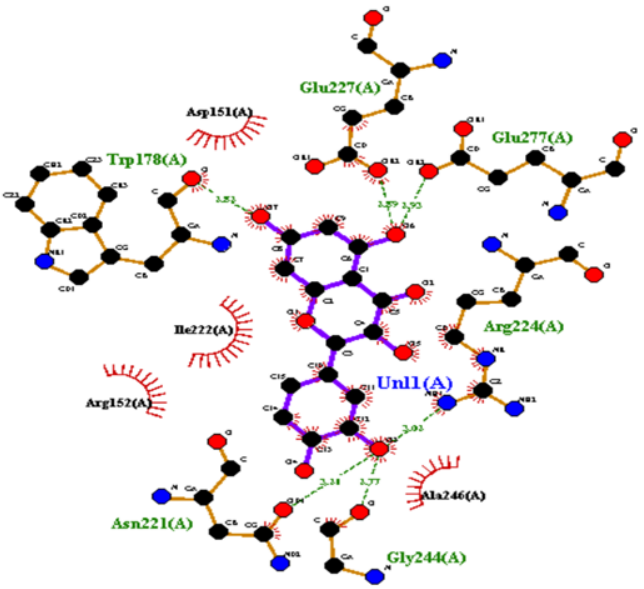


FIGURE 4.17: Interaction of Quercetin with receptor

The details of hydrogen and hydrophobic interactions are mentioned in the Table 4.34. kaempferol forms six hydrogen bonds, quercetin forms 3 whereas zanamivir form 8 hydrogen bonds, whereas zanamivir exceeds limit and violate Lipinski rule that's why kaempferol and quercetin are better.

TABLE 4.34: Docking Analysis Comparison

S.No	Ligands name	Binding Energy	No. HBs	Bonding		Hydrophobic Interactions
				Amino acids	Distance	
1	Zanamivir	-7	8	OG-Ser179- N3	3.32	Glu277
				N3-Glu227- OE2	2.8	Glu276
				OH-Tyr406- O2	2.81	Asn294
				OH-Tyr406- O3	2.82	Asp151
				NH2- Arg371- O3	3.2	Glu1
				NH1- Arg292- O3	3.1	Trp178
				NH2- Arg292- O3	3.1	Arg224
				NH2- Arg292- O4	2.81	
2	Kaempferol	-8.1	3	O6- Trp178-O	2.8	Ala246
				OE2- Glu277- O5	2.93	Arg152
				O5- Glu227- OE2	2.88	Asp151
						Arg224
						Ile222
3	Quercetin	-8.7	6	O-Gly244-O3	2.77	Ala246
				OD1-Asn221-O3	3.21	Arg152
				O3-Arg224-NH1	3.02	Ile222
				OE2-Glu277-O6	2.93	Asp151
				OE2-Glu277-O6	2.89	
				O- Trp178- O7	2.82	

The above table 4.34 shows that Glu277, Glu276, Asn294, Asp151, Glu119, Trp178, Arg224 participates in forming hydrophobic interaction between the protein and zanamivir. The oxygen atom of OG-Ser179 form bond with N3, Nitrogen atom of N3-Glu227 forms with oxygen atom of OE2.OH of Tyr406 forms bonds with oxygen atom of O2 and O3. Nitrogen of NH2- Arg371 forms hydrogen bond with O3 oxygen atom. Nitrogen of NH1- Arg292- NH2- Arg292 forms hydrogen bond with Oxygen O3, O4 between protein and zanamivir. Whereas Ala246,

Arg152, Asp151, Arg224, Ile222 participates in forming hydrophobic interaction between the protein and kaempferol. Oxygen of O6- Trp178, OE2- Glu277, O5-Glu227 forms bond with O, O5 and OE2 between Protein and kaempferol. And Ala246, Arg152, Ile222, Asp151 participates in forming hydrophobic attraction. O-Gly244-O3, OD1-Asn221-O3, O3-Arg224-NH1, OE2-Glu277-O6, OE2-Glu277-O6, O-Trp178-O7 participates in hydrogen bonding between protein and quercetin.

Chapter 5

Conclusion and Future Prospects

The study aimed to determine active constituents in the plant *Sambucus nigra* which is also known as sweet wormwood in common language. For this purpose, 10 ligands were selected to be dock against the neuraminidase of influenza virus A. The structure of all the 10 ligands was easily available in PubChem and protein structure was also available in PDB. All the ligands were docked against the receptor protein via CB Dock. The results were visualized using PyMol and were analyzed through LigPlot. Out of those 10 ligands, oleanolic acid, ursolic acid, cyanidin-3- glucoside and ethyllinoleolate were first screened out based on lipinski's rule, and based on second screening ascorbic acid, benzaldehyde, 4-vinylguaiacol and phenylacetaldehyde were knocked out. After these 2 best ligands were left Kaempferol and quercetin. Based on the hydrophobic interactions and hydrogen bonding quercetin was selected as a lead against the standard drug zanamivir which is in use for the treatment of this virus. With the final results, it was cleared that quercetin can bind far better to neuraminidase than that of zanamivir.

5.1 Recommendations

As per the findings of this research quercetin, kaempferol should be exploited more against influenza virus A. With this, other active constituent like quercetin and kaempferol have also shown a positive result in response to neuraminidase.

Previously *Sambucus nigra* has been used as anti-viral, anti-inflammatory, anti-oxidants, and anti-malarial. For this reason, *Sambucus nigra* should be explored more for its effectiveness against influenza virus A.

Bibliography

[1] P. B. Ampomah and L. H. Lim, "Influenza a virus-induced apoptosis and virus propagation," *Apoptosis*, vol. 25, no. 1, pp. 1–11, 2020.

[2] K. Urbaniak, A. Kowalczyk, and I. Markowska-Daniel, "Influenza a viruses of avian origin circulating in pigs and other mammals," *Acta Biochimica Polonica*, vol. 61, no. 3, pp. 433–439, 2014.

[3] W. Ma, "Swine influenza virus: Current status and challenge," *Virus research*, vol. 288, p. 198118, 2020.

[4] E. J. Mifsud, M. Kuba, and I. G. Barr, "Innate immune responses to influenza virus infections in the upper respiratory tract," *Viruses*, vol. 13, no. 10, p. 2090, 2021.

[5] S. Wang, B. Liang, W. Wang, L. Li, N. Feng, Y. Zhao, T. Wang, F. Yan, S. Yang, and X. Xia, "Viral vectored vaccines: design, development, preventive and therapeutic applications in human diseases," *Signal transduction and targeted therapy*, vol. 8, no. 1, p. 149, 2023.

[6] A. Jagadesh, A. A. A. Salam, P. P. Mudgal, and G. Arunkumar, "Influenza virus neuraminidase (na): a target for antivirals and vaccines," *Archives of virology*, vol. 161, pp. 2087–2094, 2016.

[7] N. M. Bouvier and P. Palese, "The biology of influenza viruses," *Vaccine*, vol. 26, pp. D49–D53, 2008.

[8] M. E. Francis, M. L. King, and A. A. Kelvin, "Back to the future for influenza preimmunity—looking back at influenza virus history to infer the outcome of future infections," *Viruses*, vol. 11, no. 2, p. 122, 2019.

[9] F. Assaad, W. C. Cockburn, and T. Sundaresan, "Use of excess mortality from respiratory diseases in the study of influenza," *Bulletin of the World Health Organization*, vol. 49, no. 3, p. 219, 1973.

[10] T. Samji, "Influenza a: understanding the viral life cycle," *The Yale journal of biology and medicine*, vol. 82, no. 4, p. 153, 2009.

[11] S. E. M. Angelena M Labella, "Influenza," *Medical clinics of North America*, vol. 97, no. 4, p. 621, 2023.

[12] J. Gong, W. Xu, and J. Zhang, "Structure and functions of influenza virus neuraminidase," *Current medicinal chemistry*, vol. 14, no. 1, pp. 113–122, 2007.

[13] A. J. Te Velthuis and E. Fodor, "Influenza virus rna polymerase: insights into the mechanisms of viral rna synthesis," *Nature Reviews Microbiology*, vol. 14, no. 8, pp. 479–493, 2016.

[14] E. C. Hutchinson, "Influenza virus," *Trends Microbiol*, vol. 26, no. 9, pp. 809–810, 2018.

[15] C.-Y. Lo, Y.-S. Tang, and P.-C. Shaw, "Structure and function of influenza virus ribonucleoprotein," *Virus protein and nucleoprotein complexes*, pp. 95–128, 2018.

[16] S. V. Vemula, J. Zhao, J. Liu, X. Wang, S. Biswas, and I. Hewlett, "Current approaches for diagnosis of influenza virus infections in humans," *Viruses*, vol. 8, no. 4, p. 96, 2016.

[17] R. J. Jackson, K. L. Cooper, P. Tappenden, A. Rees, E. L. Simpson, R. C. Read, and K. G. Nicholson, "Oseltamivir, zanamivir and amantadine in the prevention of influenza: a systematic review," *Journal of Infection*, vol. 62, no. 1, pp. 14–25, 2011.

[18] D. Rajasekaran, E. A. Palombo, T. Chia Yeo, D. Lim Siok Ley, C. Lee Tu, F. Malherbe, and L. Grollo, "Identification of traditional medicinal plant extracts with novel anti-influenza activity," *PloS one*, vol. 8, no. 11, p. e79293, 2013.

[19] G. Torabian, P. Valtchev, Q. Adil, and F. Dehghani, "Anti-influenza activity of elderberry (sambucus nigra)," *Journal of functional foods*, vol. 54, pp. 353–360, 2019.

[20] M. D. Atkinson and E. Atkinson, "Sambucus nigra l." *Journal of Ecology*, vol. 90, no. 5, pp. 895–923, 2002.

[21] T. Madej, C. J. Lanczycki, D. Zhang, P. A. Thiessen, R. C. Geer, A. Marchler-Bauer, and S. H. Bryant, "Mmdb and vast+: tracking structural similarities between macromolecular complexes," *Nucleic acids research*, vol. 42, no. D1, pp. D297–D303, 2014.

[22] N. Pagadala, K. Syed, and J. Tuszynski, "Software for molecular docking: a review. biophys rev 9: 91–102," 2017.

[23] Y. Shtyrya, L. Mochalova, and N. Bovin, "Influenza virus neuraminidase: structure and function," *Acta Naturae*, vol. 1, no. 2 (2), pp. 26–32, 2009.

[24] N. Singh, N. Anjum, and R. Chandra, "Combating influenza: natural products as neuraminidase inhibitors," *Phytochemistry Reviews*, vol. 18, pp. 69–107, 2019.

[25] F. M. Shorobi, F. Y. Nisa, S. Saha, M. A. H. Chowdhury, M. Srisuphanunt, K. H. Hossain, and M. A. Rahman, "Quercetin: a functional food-flavonoid incredibly attenuates emerging and re-emerging viral infections through immunomodulatory actions," *Molecules*, vol. 28, no. 3, p. 938, 2023.

[26] N. S. Pagadala, K. Syed, and J. Tuszynski, "Software for molecular docking: a review," *Biophysical reviews*, vol. 9, pp. 91–102, 2017.

[27] S. Hunter, P. Jones, A. Mitchell, R. Apweiler, T. K. Attwood, A. Bateman, T. Bernard, D. Binns, P. Bork, S. Burge *et al.*, "Interpro in 2011: new developments in the family and domain prediction database," *Nucleic acids research*, vol. 40, no. D1, pp. D306–D312, 2012.

[28] E. Yuriev, J. Holien, and P. A. Ramsland, "Improvements, trends, and new ideas in molecular docking: 2012–2013 in review," *Journal of Molecular Recognition*, vol. 28, no. 10, pp. 581–604, 2015.

[29] R. Dias, J. de Azevedo, and F. Walter, "Molecular docking algorithms," *Current drug targets*, vol. 9, no. 12, pp. 1040–1047, 2008.

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

[31] S. Yuan, H. S. Chan, and Z. Hu, "Using pymol as a platform for computational drug design," *Wiley Interdisciplinary Reviews: Computational Molecular Science*, vol. 7, no. 2, p. e1298, 2017.

[32] S. I. Mostafa, "Mixed ligand complexes with 2-piperidine-carboxylic acid as primary ligand and ethylene diamine, 2, 2'-bipyridyl, 1, 10-phenanthroline and 2 (2'-pyridyl) quinoxaline as secondary ligands: preparation, characterization and biological activity," *Transition Metal Chemistry*, vol. 32, no. 6, pp. 769–775, 2007.

[33] W. B. Dreitlein, J. Maratos, and J. Brocavich, "Zanamivir and oseltamivir: two new options for the treatment and prevention of influenza," *Clinical therapeutics*, vol. 23, no. 3, pp. 327–355, 2001.

[34] V. K. Morya, S. Yadav, E.-K. Kim, and D. Yadav, "In silico characterization of alkaline proteases from different species of aspergillus," *Applied biochemistry and biotechnology*, vol. 166, pp. 243–257, 2012.

[35] P. Bhattacharya and T. N. Patel, "Deregulation of mmr-related pathways and anticancer potential of curcuma derivatives–a computational approach," 2021.

[36] K. Y. Wang, F. Liu, R. Jiang, X. Yang, T. You, X. Liu, C. Q. Xiao, Z. Shi, H. Jiang, Z. Rao *et al.*, "Structure of mpro from covid-19 virus and discovery of its inhibitors," *Nature*, vol. 582, no. 7811, pp. 289–93, 2020.

[37] R. Gasparini, D. Amicizia, P. L. Lai, N. L. Bragazzi, and D. Panatto, "Compounds with anti-influenza activity: present and future of strategies for the optimal treatment and management of influenza part ii: Future compounds against influenza virus," *Journal of preventive medicine and hygiene*, vol. 55, no. 4, p. 109, 2014.

[38] Y. Furuta, K. Takahashi, Y. Fukuda, M. Kuno, T. Kamiyama, K. Kozaki, N. Nomura, H. Egawa, S. Minami, Y. Watanabe *et al.*, "In vitro and in vivo activities of anti-influenza virus compound t-705," *Antimicrobial agents and chemotherapy*, vol. 46, no. 4, pp. 977–981, 2002.

[39] N. I. Okasha, M. A. Rahman, M. S. Nafie, N. M. A. Shama, A. Mostafa, D. A. El-Ebeedy, and A. Z. A. Azeiz, "Identification of potential antiviral compounds from egyptian sea stars against seasonal influenza a/h1n1 virus," *Journal of Genetic Engineering and Biotechnology*, vol. 22, no. 1, p. 100334, 2024.

[40] G. Marsili, C. Acchioni, A. L. Remoli, D. Amatore, R. Sgarbanti, M. De Angelis, R. Orsatti, M. Acchioni, A. Astolfi, N. Iraci *et al.*, "Identification of anti-influenza a compounds inhibiting the viral non-structural protein 1 (ns1) using a type i interferon-driven screening strategy," *International Journal of Molecular Sciences*, vol. 24, no. 13, p. 10495, 2023.

[41] M. M. Mia, M. Hasan, M. M. Miah, M. A. S. Hossain, S. E. V. T. S. M. S. Islam, and V. C. P. H. R. N. Shanta, "Inhibitory potentiality of secondary metabolites extracted from marine fungus target on avian influenza virus-a subtype h5n8 (neuraminidase) and h5n1 (nucleoprotein): a rational virtual screening," *Veterinary and Animal Science*, vol. 15, p. 100231, 2022.

[42] ——, "Inhibitory potentiality of secondary metabolites extracted from marine fungus target on avian influenza virus-a subtype h5n8 (neuraminidase) and h5n1 (nucleoprotein): a rational virtual screening," *Veterinary and Animal Science*, vol. 15, p. 100231, 2022.

[43] C. Zhang, J. Xiang, Q. Xie, J. Zhao, H. Zhang, E. Huang, P. Shaw, X. Liu, and C. Hu, "Identification of influenza pan endonuclease inhibitors via 3d-qsar modeling and docking-based virtual screening," *Molecules*, vol. 26, no. 23, p. 7129, 2021.

[44] M. A. Zeller, P. C. Gauger, Z. W. Arendsee, C. K. Souza, A. L. Vincent, and T. K. Anderson, "Machine learning prediction and experimental validation of

antigenic drift in h3 influenza a viruses in swine," *Msphere*, vol. 6, no. 2, pp. 10–1128, 2021.

[45] P. Hariyono, J. C. Kotta, C. F. Adhipandito, E. Aprilianto, E. J. Candaya, H. A. Wahab, and M. Hariono, "A study on catalytic and non-catalytic sites of h5n1 and h1n1 neuraminidase as the target for chalcone inhibitors," *Applied Biological Chemistry*, vol. 64, pp. 1–17, 2021.

[46] S. Bangaru, P. Manivannan, and S. Muthu, "Spectroscopic investigations, quantum chemical calculations and molecular docking studies of mangiferin-an anti-viral agent of h1n1 influenza virus," *Chemical Data Collections*, vol. 30, p. 100580, 2020.

[47] Y. Ren, S. Long, and S. Cao, "Molecular docking and virtual screening of an influenza virus inhibitor that disrupts protein–protein interactions," *Viruses*, vol. 13, no. 11, p. 2229, 2021.

[48] S. Sasidharan, V. Gosu, D. Shin, S. Nath, T. Tripathi, and P. Saudagar, "Therapeutic p28 peptide targets essential h1n1 influenza virus proteins: insights from docking and molecular dynamics simulations," *Molecular Diversity*, vol. 25, pp. 1929–1943, 2021.

[49] M. Sahoo, L. Jena, S. N. Rath, and S. Kumar, "Identification of suitable natural inhibitor against influenza a (h1n1) neuraminidase protein by molecular docking," *Genomics & informatics*, vol. 14, no. 3, p. 96, 2016.

[50] A. Ahmad, A. Ahad, A. Q. Rao, and T. Husnain, "Molecular docking based screening of neem-derived compounds with the ns1 protein of influenza virus," *Bioinformation*, vol. 11, no. 7, p. 359, 2015.

[51] S. Bangaru, G. Madhu, M. Srinivasan, and P. Manivannan, "Exploring flexibility, intermolecular interactions and admet profiles of anti-influenza agent isorhapontigenin: A quantum chemical and molecular docking study," *Heliyon*, vol. 8, no. 8, 2022.

[52] V. Karthick, K. Ramanathan, V. Shanthi, and R. Rajasekaran, "Identification of potential inhibitors of h5n1 influenza a virus neuraminidase by ligand-based

virtual screening approach," *Cell biochemistry and biophysics*, vol. 66, pp. 657–669, 2013.

[53] S. D. Ghate, L. Pinto, S. Alva, M. G. Srinivasa, R. K. Vangala, P. Naik, B. Revanasiddappa, and R. S. P. Rao, "In silico identification of potential phytochemical inhibitors for mpox virus: molecular docking, md simulation, and admet studies," *Molecular Diversity*, pp. 1–20, 2024.

[54] S. S. Mohseni, F. Nasri, K. Davari, S. Mirzaie, A. Moradzadegan, F. Abdi, and F. Farzaneh, "Identification of novel inhibitor against endonuclease subunit of influenza ph1n1 polymerase: A combined molecular docking, molecular dynamics, mmpbsa, qmmm and adme studies to combat influenza a viruses," *Computational Biology and Chemistry*, vol. 77, pp. 279–290, 2018.

[55] D. Slain, "Intravenous zanamivir: a viable option for critically ill patients with influenza," *Annals of Pharmacotherapy*, vol. 55, no. 6, pp. 760–771, 2021.

[56] M. Abdullahi, A. Uzairu, G. A. Shallangwa, P. A. Mamza, and M. T. Ibrahim, "Modelling of novel bornoel analogs as influenza a virus inhibitors through genetic function approximation, comparative molecular fields, molecular docking, and admet/pharmacokinetic studies," *Intelligent Pharmacy*, vol. 2, no. 2, pp. 190–203, 2024.

[57] M. Dammalli, V. Chandramohan, M. I. Biradar, N. Nagaraju, and B. S. Gangadharappa, "In silico analysis and identification of novel inhibitor for new h1n1 swine influenza virus," *Asian Pacific Journal of Tropical Disease*, vol. 4, pp. S635–S640, 2014.

[58] S. Bhakat, F. Shaikh, S. Yadav, and R. M. Rawal, "Identification of neuraminidase inhibitors by structure-based screening: promising new leads for influenza," *Medicinal Chemistry Research*, vol. 23, pp. 2803–2809, 2014.

[59] D. K. Behera, P. M. Behera, L. Acharya, A. Dixit, and P. Padhi, "In silico biology of h1n1: molecular modelling of novel receptors and docking studies of inhibitors to reveal new insight in flu treatment," *BioMed Research International*, vol. 2012, no. 1, p. 714623, 2012.

[60] L. Birch, C. W. Murray, M. J. Hartshorn, I. J. Tickle, and M. L. Verdonk, "Sensitivity of molecular docking to induced fit effects in influenza virus neuraminidase," *Journal of Computer-Aided Molecular Design*, vol. 16, pp. 855–869, 2002.

[61] E. De Clercq, "Antiviral agents active against influenza a viruses," *Nature reviews Drug discovery*, vol. 5, no. 12, pp. 1015–1025, 2006.

[62] D. Lee, H. Jo, Y. Jang, S. Bae, T. Agura, D. Kang, M. Kang, Y. Kim, N.-H. Cho, Y. Kim *et al.*, "Alloferon and zanamivir show effective antiviral activity against influenza a virus (h1n1) infection in vitro and in vivo," *International Journal of Molecular Sciences*, vol. 24, no. 1, p. 678, 2022.

[63] J. S. Nguyen-Van-Tam, "Zanamivir for influenza: a public health perspective: Its use will require careful management by gps," pp. 655–656, 1999.

[64] C. J. Heneghan, I. Onakpoya, M. Thompson, E. A. Spencer, M. Jones, and T. Jefferson, "Zanamivir for influenza in adults and children: systematic review of clinical study reports and summary of regulatory comments," *Bmj*, vol. 348, 2014.