IN SILICO INTERVENTION TO DELINEATE FLAVONOIDS TARGETING NSP14 AND NSP 15 OF SARS- CoV-2

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Submitted by:

Khyati Rastogi

2K21/MSCBIO/19

Under the supervision of:

Dr. NAVNEETA BHARADVAJA

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi – 110042

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DEPARTMENT OF BIOTECHNOLOGY (DTU)

CANDIDATE'S DECLARATION

I, Khyati Rastogi , Roll Number: 2K21/MSCBIO/19, student of M.Sc. Biotechnology, hereby declares that the work which is presented in the Major Project entitled "**INSILICO INTERVENTION TO DELINEATE FLAVONOIDS TARGETING NSP14 AND NSP 15 OF SARS- CoV-2**" in the fulfillment of the requirement for the award of the degree of Master of Science in Biotechnology and submitted to the Department of Biotechnology, DTU, is an authentic record of my own carried out during the period from Jan to May 2023, under the supervision of **Dr. Navneeta Bharadvaja**. I have not applied for any other degree at this or any other University based on the information contained in this report. The following details about the related study have been approved in the IEEE Conference:

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To the best of my knowledge, the work titled "**SILICO INTERVENTION TO DELINEATE FLAVONOIDS TARGETING NSP14 AND NSP 15 OF SARS- CoV-2**" has not been submitted anywhere else either in part or in full for any Degree or Diploma at this University or elsewhere. I further certify that the publication and indexing information given by the student is correct.

Dr. Navneeta Bharadvaja
SUPERVISIOR
Assistant Professor
Department of Biotechnology
Delhi Technological University

Prof. Pravir Kumar

Head of Department
Department of Biotechnology
Delhi Technological University

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KHYATI RASTOGI

2K21/MSCBIO/19

ABSTRACT

The SARS-CoV-2 global pandemic has resulted in a serious medical emergency that is still affecting people all over the world. Since its discovery in December 2019, COVID-19 has spread quickly through direct contact with people and respiratory aerosols. The research targeted two important SARS-CoV-2 virus proteins, NSP 14 and NSP 15, with the goal of finding flavonoids that could potentially function as antagonists, thus preventing immune-mediated reactions, in order to treat the harmful impacts of this infectious disease.

To achieve this, a group of 28 flavonoids were evaluated using in silico molecular docking and drug-likeness testing methodologies. It is notable that the flavonoids discovered through computational predictions as potential SARS-CoV-2 antagonists have already been linked to a number of therapeutic advantages. The COCONUT database and academic publications were used to find these flavonoids. To find flavonoids that demonstrated efficient binding to the target proteins NSP 14 and NSP 15, a computational analysis method was used. Two remarkable candidates—Silymarin and Bilobetin—were identified among the flavonoids examined. These substances demonstrated strong interactions with NSP 14 and NSP 15 which were characterised by extended close contact and minimal binding energies of -7.45 and -8.03 kcal/mol, respectively. Silymarin and bilobetin have been found to have a high affinity for the targeted proteins, which suggests that they could be effective antagonists. Additionally, the computational strategy used in this study offers a useful tool for quickly screening and finding therapeutic drug candidates. Large compound libraries can be evaluated using in silico molecular docking, which cuts the time and expense of experimental screening. We can speed up the drug development process and choose the most

promising candidates for additional research by integrating these computational tools with current information of flavonoid characteristics and their claimed advantages.

Keywords— Flavonoids, SARS-CoV-2, Nsp14, Nsp 15, Molecular Docking, Toremifene, Tipiracil, Silymarin, Bilobetin.

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CHAPTER 1

INTRODUCTION

1.1Background

A significant medical emergency brought on by the SARS-CoV-2 worldwide pandemic is still having an impact on individuals all over the world. COVID-19 was found in December of last year. Millions of infections and fatalities have been brought on by the virus that caused the coronavirus disease 2019 (COVID-19) pandemic worldwide. SARS-CoV-2, formerly known as the 2019 novel coronavirus (2019-nCoV), has genetic traits that are unique but like those of other coronaviruses[1],[2]. SARS-CoV-2 is larger than many other RNA viruses, with an RNA genome that ranges in size from 27 to 32 kilobases[3]. The four structural proteins identified by the letters membrane (M), spike (S), envelope (E), and nucleocapsid (N) are present in the virus. 16 non-structural proteins (Nsp1-16) and eight accessory proteins are also present. While structural proteins are important for transcription and replication, they also play a role in the development of new virions.

SARS-CoV-2's relatively big genome compared to other RNA viruses is one of its defining features[4].Numerous proteins that are necessary for the virus's transcription, reproduction, and interaction with host cells are found in its genome.

The SARS-CoV-2 viral genome is made up of 16 non-structural proteins (nsp1–16), 4 structural proteins, and 8 accessory proteins. The generation of new virions is essential for the viral development process, but viral transcription and replication depend on non-structural proteins. Important functions

performed by these proteins include modifying host cell processes, eluding the host immune system, and reproducing viral RNA. Because of their importance in viral transcription and replication, a few Nsps, notably Nsp3, Nsp5, and Nsp12, have been singled out for future research as antiviral treatment targets. It is essential to comprehend the genetic structure and protein composition of SARS-CoV-2 in order to create efficient diagnostic procedures, treatments, and vaccinations[5], [6].Scholars and experts from across the world have been studying the virus in detail to understand how it spreads and how it causes disease. This information lays the groundwork for the creation of plans to stop the virus' propagation and care for COVID-19 victims.

1.2 Significance of Study

This study's significance stems from its analysis of the functions of Nsp14 and Nsp15, two crucial proteins, in the replication and pathogenesis of SARS-CoV-2, the virus that gave rise to the COVID-19 pandemic. It may be possible to create specialized treatment strategies by comprehending the roles and workings of certain proteins. An N-terminal exonuclease and a C-terminal N7-methyltransferase are two different effective domains of the SARS-CoV-2 protein Nsp14, which is involved in replication. A crucial protein involved in the replication of SARS-CoV-2 is non-structural protein 14 (Nsp14). The function of Nsp14 in viral replication is discussed in this article, and its potential as a therapeutic target is emphasized[7]. Proofreading procedure, which aids in preserving the genetic integrity of the viral genome during replication, is carried out by the N-terminal exonuclease. Nsp14 lowers the rate of mutagenesis, allowing the virus to maintain stability and spread effectively by identifying and fixing errors in the viral RNA.

The evolution and control of coronaviruses with large genomes depend heavily on Nsp14's proofreading function. SARS-CoV-2's enormous genome size creates an issue for replication fidelity. The virus's capacity to adapt to host and environmental changes while maintaining genetic stability is aided by Nsp14's proofreading activity. This mechanism might account for the SARS-CoV-2 virus's relatively low mutation rate when compared to other RNA viruses[8].

Nsp14 is potential target for therapeutic interventions because of its crucial function in preserving the genetic stability of SARS-CoV-2. The N-terminal exonuclease domain of Nsp14 has attracted a lot of interest as a potential drug target. It may be possible to cause severe mutagenesis in the viral genome and make it nonviable or significantly less virulent by inhibiting Nsp14's proofreading function[9].

An intriguing candidate for converting against SARS-CoV-2 is toremifene, an FDA-approved medication for metastatic breast cancer. Since toremifene has a high affinity for Nsp14, it is a desirable control for future drug development projects. Toremifene and other potential inhibitors may interfere with the proofreading procedure by targeting Nsp14, causing the viral genome to accumulate mutations and obstructing viral replication[10], [11].

Despite the promise of Nsp14 as a target for drug development, more studies and clinical experiments are required to determine the effectiveness and security of such interventions. It is crucial to consider any potential resistance mechanisms that might develop as a result of repeated exposure to Nsp14 inhibitors. Additional investigation is needed to fully

recognise the structural and functional properties of Nsp14, which will help in the creation of effective and targeted inhibitors. Nsp14 has been linked to other viral life cycle processes, including pathogenicity, genomic recombination, and innate immune responses, in addition to its function in replication fidelity.

Investigating Nsp14's additional capabilities may reveal more details about the behaviour of the virus and potential therapeutic intervention targets. Nsp15 contributes significantly to coronavirus pathogenesis by destroying intermediate forms of the viral double-stranded RNA (dsRNA). When this degradation occurs, the host is blocked from identifying dsRNA, which sets off the innate immune response. The virus can successfully infect the host by evading immune defences by avoiding detection. Nsp15 has consequently become a possible target for therapeutic intervention against coronaviruses[12].

The unique properties and essential functions of Nsp15 make it a suitable candidate for the development of COVID-19 therapeutics. Nsp15 exhibits endoribonuclease activity and clearly prioritises uridine bases when cleaving RNA substrates at the 3' end of pyrimidines. It demonstrates a tendency for cleaving unpaired uridine nucleotides in RNAs with a hairpin shape. The RNA substrate's secondary structure can affect Nsp15 activity, with hairpin structures facilitating cleavage. Nsp15 activity has been reported to be blocked RNA substrate containing 2'-O-ribose methyl groups[13]. Three dimers make up the 234 kDa hexameric enzyme known as Nsp15. It is unique among RNA viruses due to its sequence and structural characteristics, which designate it as a member of the Nidovirales order[14]. Although distant homologs (XendoU) have been discovered in some prokaryotes and eukaryotes, their main function is the processing of small nucleolar RNAs that are encoded by introns[15].

Nsp15 is a promising target for the creation of COVID-19 therapeutics due to its distinct properties and essential function in coronavirus pathogenesis. The process of drug repurposing has been sped up by the discovery of FDA-approved medications that may have inhibitory effects on Nsp15. Tipiracil is one such medication, used to treat colorectal cancer. Tipiracil associates through uridine binding pocket located within the active site of the enzyme to inhibit SARS-CoV-2 Nsp15[16], [17].

This interaction prevents the enzyme from doing its job and prevents the virus from replicating and becoming pathogenic.

The treatment of cases of the global COVID-19 crisis may be aided by natural substances derived from plants, particularly those in the flavonoid family[18]. Flavonoids are appealing candidates for halting the SARS-CoV-2 virus' life cycle because they have antiviral, antioxidant, and anti-inflammatory properties[19]. Flavonoids are more appealing as potential therapeutic options because they do not exhibit systemic toxicity, in contrast to conventional medications.

The COCONUT (Collection of Open Natural Products) database is an excellent resource to look for information on flavonoids. An online open-source project called COCONUT is intended to store, search for, and examine natural products. disease.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of SARS-CoV-2 and COVID-19

A severe acute respiratory syndrome, A novel coronavirus called Coronavirus 2, or SARS-CoV-2, was discovered in the Chinese city of Wuhan in the latter half of the year. Members of the same viral family include the previous SARS-CoV and MERS-CoV viruses as well as this one. A major impact has been made on people's health, economies, and cultures all around the world by the COVID-19 pandemic, which was brought on by the SARS-CoV-2 virus.

Droplets of respiratory fluid from an infected people are essentially how SARS-CoV-2 is disseminated when the individual sneezes, coughs, speaks, or breathes[20].

Touching contaminated surfaces before contacting your face can spread it. The virus primarily targets the respiratory system, and to enter human cells and spread infection, it has a spike protein on its surface that binds to receptors, notably in the lungs. Once within the body, the virus multiplies in the respiratory system, resulting in a spectrum of symptoms that can be mild to severe in intensity. Some of the most prevalent COVID-19 symptoms are throat discomfort, a high body temperature coughing, difficulty of breath, tiredness, muscular pains, and a diminished sense of taste or smell in extreme instances, pneumonia and the acute respiratory distress syndrome (ARDS) might develop, demanding hospitalisation and critical care[21].

All ages are at risk from COVID-19, but older persons and those who already have medical conditions are more likely to suffer significant consequences[22]. There are many different clinical manifestations of the condition, with some people being asymptomatic or having little symptoms while others go through severe illness and even pass away. To curb the spread of SARS-CoV-2, a number of public health measures have been implemented worldwide, including the use of face masks, physical separation, regularly cleaning of hands, and lockdowns or restrictions on public gatherings. Vaccination initiatives have started, which offer defence against the virus and lessen the severity of disease.

Global efforts are being made to create COVID-19 therapies and vaccinations. A number of vaccinations have been approved for use in emergency situations and have demonstrated encouraging outcomes in lowering the severity of sickness, averting hospitalisation, and preventing mortality[23].

2.2 Structure and Function of NSP14

NSP14 is a versatile protein that plays a variety of roles in viral replication because to different domains. It is made up of a C-terminal N7-methyltransferase domain that methylates the viral RNA cap and an N-terminal exonuclease domain that performs proofreading[24]. For the viral genome to remain intact during replication, NSP14's proofreading activity is essential[25]. It fixes errors that happen during RNA production, which lowers the mutation rate and promotes genome stability. Despite having huge

genomes, coronavirus replication has a high level of fidelity, which can be linked to NSP14's proofreading function.

The N7-methyltransferase domain of the NSP14 protein is responsible for methylation of the RNA cap structure. The stability of viral mRNA, evasion of host immunological responses, and effective translation of viral proteins all depend on this alteration. The methylation cap structure also aids in the separation of viral from host RNA, which facilitates the packaging and export of viral transcripts with more specificity. Therefore, NSP14's N7-methyltransferase activity supports coronavirus pathogenicity and effective replication[26].

2.3 Structure and Function of NSP15

Only viruses belonging to the order Nidovirales contain the conserved uridine-specific endoribonuclease known as nsp15. There were only distant homologs (XendoU) for the prokaryotes and eukaryotes that have been identified to be involved in the processing of intron-encoded short nucleolar RNAs. Three dimers make up the 234 kDa hexameric enzyme known as Nsp15. It is unique among RNA viruses due to its sequence and structural characteristics, which designate it as a member of the Nidovirales order. Although distant homologs (XendoU) have been discovered in some prokaryotes and eukaryotes, their main function is the processing of small nucleolar RNAs that are encoded by introns[27], [28].

The principal function of NSP15 is to cleave RNA substrates due to its endonucleolytic activity. A molecule with 2' to 3' cyclic phosphate and 5' hydroxyl ends is released as a result of the specific 3' end of pyrimidines in RNA being cleaved. Because enzymes prefer to cleave unpaired uridine bases in RNAs with hairpin structures, NSP15's activity is regulated

by the secondary structure of the RNA substrate. Because of this cleavage pattern, it is possible that NSP15 is involved in the processing of structured RNA sections seen inside viral genomes.

2.4 Flavonoids and their Potential Therapeutic Benefits

The treatment of severe cases of the global COVID-19 crisis may be aided by natural compounds produced from plants, particularly those in the flavonoid family. Flavonoids are appealing possibilities for halting the SARS-CoV-2 virus' life cycle because they include antiviral, antioxidant, and anti-inflammatory characteristics. Flavonoids are appealing as prospective therapeutic choices because they do not demonstrate systemic toxicity, in contrast to conventional pharmaceuticals. Flavonoids are a broad class of substances that are widely distributed in a variety of fruits, vegetables, herbs, and other plant-based sources[29], [30]. Their therapeutic qualities and potential for improving health have been thoroughly investigated. Considering that flavonoids exhibit a variety of biological functions, including antiviral characteristics, they are particularly fascinating in the context of treating COVID-19. A strong immune system is supported by flavonoids as well. They have antiviral and antibacterial effects, can boost the activity of immune cells, and encourage the formation of antibodies. Flavonoids help to overall health and infection resistance by boosting the immune system[31].

TABLE 1 – LIST OF FLAVONOIDS USED IN MOLECULAR DOCKING ANALYSIS

Engelitin	Cosmosiin	Licoagrocarpin
Miquelianin	Cosmosiinnoxerutin	Licoagrochalcone A
ambocein	Narcissoside	Karanjin
diosmin	Nepitrin	Isorhoifolin
Isoorientin	Galangin	Iristectorigenin B
Bilobetin	Silibrinin	Hibifoline
molnupiravir	Limocitrin	Cianidano
Rutin	Pinocembrin	Chrysin

2.5 Previous Research on Flavonoids and SARS-CoV-2

Two flavonoids, quercetin and epigallocatechin gallate (EGCG), have drawn a lot of interest because of their potential to fight COVID-19. Numerous fruits and vegetables contain a chemical called quercetin, which has been shown to have antiviral activity against a variety of viruses, including coronaviruses. According to studies, quercetin prevents viral proteins and enzymes necessary for the virus's life cycle from entering cells and replicating. Green tea's rich EGCG has similar antiviral effects on a number of viruses. It has been discovered to prevent viral entry and attachment as well as to obstruct viral reproduction procedures[32].

Other flavonoids, including hesperetin, apigenin, and baicalein, have also demonstrated promise in preventing viral replication and minimising the inflammation brought on by viral infections. These flavonoids have a variety of molecular configurations that give them the ability to engage with viral proteins and obstruct viral reproduction processes[33].

2.6 Current Treatment Options for COVID-19

The current COVID-19 therapy regimen includes the administration of antiviral drugs such Remdesivir, Hydroxychloroquine, Favipiravir, and different steroids. However, their effectiveness and safety have come under criticism because of a number of undesirable consequences. These medications may not regularly be powerful enough to treat dangerous infections, according to research. Additionally, several countries have discontinued utilising certain medicines due to concerns about their effectiveness and inappropriate use, such as plasma therapy employing monoclonal antibodies[34], [35].

Early in the epidemic, hydroxychloroquine, a drug often used to treat autoimmune diseases and malaria, received a lot of attention, but its effectiveness and safety have come into doubt. Although there was initially a lot of enthusiasm based on in vitro investigations, later clinical trials have not consistently been able to demonstrate advantages. The World Health Organisation (WHO) terminated its Solidarity Trial investigating hydroxychloroquine because to concerns regarding its safety and inefficiency in treating COVID-19[35].

Favipiravir, an antiviral drug currently approved for the treatment of influenza in some nations, has also being investigated as a possible COVID-19 therapy. Favipiravir functions as a purine analogue and inhibits viral replication when used in lieu of guanine or adenine. Its therapeutic efficacy for a number of illnesses has been demonstrated. Its therapeutic

usefulness for a number of illnesses has been proven. Lassa fever, Ebola, and other illnesses that pose a serious risk to life have all been treated with it. More research is needed to determine whether it is appropriate for the treatment of COVID-19[36], [37].

The management of severe COVID-19 cases has shown promise when using steroids, such as dexamethasone. Due to their anti-inflammatory qualities, steroids can help lessen lung inflammation and complications brought on by the immune system. The creation and distribution of COVID-19 vaccines has been a key area of focus in the fight against the pandemic, in addition to pharmaceutical interventions. Covishield (Oxford-AstraZeneca) and Sputnik V (Gamaleya Research Institute) are two vaccine candidates that have been introduced and given emergency use approval in several nations[38].

The existence of new variants has been caused by the ongoing mutations in the SARS-CoV-2 genome, raising questions about the efficacy of the available vaccines Variants like the Alpha, Beta, Gamma, and Delta variants have shown increased infectiousness and, in certain circumstances, the ability to partially avoid immune responses. This has brought attention to the necessity of ongoing research, surveillance, and potential vaccine adaptations to address new variants[39].

2.7 Overview of Tipiracil and Toremifene (Control)

It has been proven that toremifene, an FDA-approved drug that was first intended to treat metastatic breast cancer, can be used to treat COVID-19, the virus that causes it. Toremifene, a non-steroidal substance, received its initial approval in 1997 as a selective oestrogen receptor modulator. Researchers discovered toremifene as a top candidate for the treatment of COVID-19 through a network medicine study[40]. At concentrations in the micromolar

range, toremifene has been shown in laboratory studies to be able to inhibit several viral infections, including the Middle East Respiratory Syndrome coronavirus (MERS-CoV), the severe acute respiratory syndrome coronavirus (SARS-CoV), and SARS-CoV-2[41]. The idea that toremifene might obstruct the interaction between ACE2 and the spike protein of SARS-CoV-2 was further validated by computational biophysical investigations. When a virus infects human cells, it does so via the ACE2 receptor. Toremifene might prevent this interaction and obstruct viral entrance into host cells, hence reducing viral reproduction[42]. The computer research also suggested that toremifene might possibly prevent the action of a particular viral protein called non-structural protein 14 (nsp14), which is crucial in viral reproduction. Toremifene might interfere with the viral life cycle and make it more difficult for the virus to spread by specifically targeting nsp14.Toremifene fights SARS-CoV-2 through a variety of pathways. It is important to remember that additional study, including clinical trials, would be required to demonstrate toremifene's efficacy and safety as a COVID-19 medication. Finding viable treatments for new indications like COVID-19 more quickly may be possible by repurposing currently available medications, such as toremifene. Researchers can hasten the process of developing new therapeutics and look at potential treatments for the ongoing epidemic by utilising the expertise and safety profiles of FDA-approved medications. However, before any medication can be utilised generally for the treatment of COVID-19, it must first undergo a thorough examination and confirmation[43].

Tipiracil, an FDA-approved medication predominantly used to treat colorectal cancer, has been investigated as a potential anti-COVID-19 medicine in recent investigations[44]. Researchers have studied the interaction between Tipiracil and a particular enzyme in SARS-CoV-2 known as Nsp15 using a variety of scientific techniques including

crystallography, biochemical assays, and whole-cell assays. Tipiracil was shown to inhibit Nsp15, but this was not enough to declare it a COVID-19 treatment that worked. The finding of this interaction, however, sheds light on Tipiracil's potential as a basis for the creation of fresh substances that might successfully stop the spread of the virus. According to the results of these investigations, Tipiracil can interfere with Nsp15 activity by interacting with a particular pocket in the enzyme's active site that usually binds uridine. Nsp15, which is known to be involved in viral replication, is unable to function normally as a result of this interaction[45].

In addition, thymidine phosphorylase, another enzyme essential for the metabolism of trifluridine, is known to be inhibited by tipiracil. The possible antiviral benefits of Tipiracil may be indirectly attributed to this inhibitory mechanism.Tipiracil can operate as a scaffold or template for the creation and synthesis of modified compounds that might have improved inhibitory efficacy against Nsp15 or other viral targets. These substances may be transformed into stronger antiviral medications to combat COVID-19. It is significant to emphasise that additional investigation, including clinical studies, would be required to ascertain the effectiveness and security of tipiracil or any derived compounds as COVID-19 therapies. Drug development can be sped up by using FDA-approved medications for novel purposes, but careful review and validation are required to make sure they are effective against the unique mechanisms of the SARS-CoV-2 virus[46].

CHAPTER 3

TOOLS AND MATERIAL

Elevated Various resources and tools were used in the current study to collect and analyze data. In addition to bioinformatics tools and biological databases like PubMed, PubChem, and the Protein Data Bank (PDB), they featured software programmes including Biovia Discovery Studio, UCSF Chimaera, and Swiss Dock.

PubMed is a resource for discovering research and review papers in the biomedical and life sciences sectors and is run by the National Centre for Biotechnology Information (NCBI) at the National Institutes of Health (NIH). Researchers may use it as a free search engine to trawl through a range of scientific publications and stay current on advancements in their disciplines[47].

In comparison, PubChem is a well-known open database of chemical compounds. It enables researchers to look up specific chemical names, learn more about the properties and structures of such compounds, and investigate the biological processes connected to them. Researchers researching at the potential medical benefits of various chemical compounds might benefit from PubChem. The Protein Data Bank (PDB) initiative makes it feasible to comprehend the structural characteristics of proteins, nucleic acids, and complex compounds, which is crucial for comprehending how they interact and operate.

To make it simpler to analyse protein structures and ligand docking studies, a few software tools were employed, including Swiss Dock, UCSF Chimaera, and Biovia Discovery Studio.

The computer tools **Swiss Dock and Swiss Dock ADME** are utilized in the drug research and development domains. They are designed to aid scientists in predicting the interactions between minute compounds (such potential drug candidates) and biological targets as well as in estimating the characteristics of these molecules' Absorption, Distribution, Metabolism, and Excretion (ADME). Both algorithms, which offer significant insights into the molecular principles behind drug action and pharmacokinetics, were developed at the Swiss Institute of Bioinformatics (SIB).

A platform for interactive visualization and analysis of molecular structures is offered by UCSF Chimaera, another piece of software. Software programmes called Swiss Dock and Swiss Dock ADME were used especially for ligand docking studies. They help predict ligand-receptor interactions and evaluate drug ADME (absorption, distribution, metabolism, and excretion) properties. With the aid of computer methods, Swiss Dock, a molecular docking programme, simulates and forecasts the binding interactions between a tiny molecule (the ligand) and a target protein (the receptor). It explores the conformational space of the ligand and receptor and calculates the binding affinity using a combination of molecular mechanics force fields, search algorithms, and scoring functions. The virtual screening and lead optimization processes have been greatly facilitated by Swiss Dock, which enables researchers to find prospective drug candidates with excellent binding affinity and selectivity for a particular target. Numerous studies have shown SwissDock to be accurate and reliable, making it an important tool in the early phases of drug discovery. These methods are essential for comprehending the molecular interactions that take place between ligands and proteins, foretelling the potential effectiveness and safety of substances, and ultimately for assisting in the creation of novel therapeutic interventions

CHAPTER 4

METHODOLOGY

4.1 Preparation and Refinement of Protein (NSP14 and NSP 15)

The Protein Data Bank (PDB) was used to obtain the proteins NSP14 (7R2V) and NSP15 for additional examination and improvement. Biovia Discovery Studio software was used to enhance these proteins' structural integrity. The quality of the protein structures was improved through several procedures.

Using Biovia Discovery Studio, water molecules, ligands, and heteroatoms that were present in the protein structures were first eliminated. Although significant in their respective biological contexts, these elements were left out of the analysis so that the protein of interest could be the only thing examined. The structure and purpose of the protein were better understood as a result of this step.

The protein structures were also modified with Biovia Discovery Studio to include non-polar hydrogens. When analysing the overall stability and interactions of proteins, hydrogens are an important factor. Non-polar hydrogens were used to better represent the refined protein structures, considering their hydrophobic regions and interactions. For charge computation, the Gasteiger method was used to further improve the protein structures. This approach was aided by the Autodock Tools in Biovia Discovery Studio[48]. Studying the behaviour of the protein, especially in connection to ligand binding and molecular interactions, requires accurate determination of the partial charges on the atoms.

Biovia Discovery Studio was used to locate the proteins' active areas. Proteins that include active sites carry out vital biological processes like ligand binding and enzymatic reactions. The discovery of these active regions offers important insights into the workings of the

protein and its interactions with other molecules. The finalised protein structures were saved in the PDB format when the preparation and refinement processes were finished. Protein structures can be easily shared, visualised, and subjected to additional investigation thanks to this format, which is widely used in structural biology.

4.2 Preparation of flavonoids as ligand library

A number of procedures were followed in the creation of a ligand library of flavonoids. First, the structures of the FDA-approved drugs, Toremifene (PubChem ID: 3005573) and Tipiracil (PubChem ID: 6323266), were retrieved from the PubChem database. A variety of flavonoids were also chosen to be included in the library. The SDF (Structure-Data File) format, a common file format for storing chemical structures and related data, was used to download these structures.

The Biovia discovery studio was used to make it easier to analyse and use these structures going forward. This software programme provides a wide range of capabilities for research on drug development and molecular modelling. One of the critical phases in developing the ligand library was converting the obtained structures from SDF to Mol2 format. The Mol2 file format, which additionally contains information on atom types, bond connectivity, and partial charges, is commonly used in molecular modelling.

The transformed structures are made compatible with a wide range of tools and applications by being converted to the Mol2 file format. This conversion process was aided by the Biovia discovery studio, which made sure that the structures were modified precisely while preserving their important molecular characteristics. This process was necessary to

guarantee the smooth incorporation and compatibility of the flavonoids and FDA-approved medicines into the ligand library.

The decision to include FDA-approved medications in the control group had major implications. FDA-approved medications have gone through extensive testing and approval procedures, making them excellent benchmarks for comparison and validation in drug development research. As representatives from this group, Toremifene and Tipiracil were particularly chosen, and the PubChem database was used to acquire their chemical structures. Due to their presence in the ligand library and their established efficacy and safety profiles, these medications serve as a standard by which the flavonoids can be measured. Exploration of their biological activities and interactions in drug discovery was made possible by the integration of flavonoids, which expanded the diversity and potential of the library format.

4.3 Molecular Docking

PDB The interaction between a protein and a ligand molecule is studied using the computer method known as molecular docking. SwissDock, a popular docking programme (available at http://www.swissdock.ch/), was used in this study to conduct molecular docking studies. Proteins must be in the Protein Data Bank (PDB) format and ligands must be in the Mol2 format for SwissDock to work.

The target protein was docked with Toremifene and Tipiracil to start the investigation. These drugs were chosen as the control substances. The binding energy between each medication and each protein was determined and recorded during the docking procedure. Additionally, UCSF Chimaera (http://www.cgl.ucsf.edu/chimera/) and Biovia Discovery Studio were

used to create the 3D and 2D diagrams of the protein-ligand interactions in order to visualize the binding modes.

These technologies allowed us to create intricate structural diagrams showing the three-dimensional organization of the protein-ligand complexes. The 3D models revealed crucial interactions such as hydrogen bonds, hydrophobic interactions, and electrostatic interactions, which shed light on how the ligands connect to the protein. Understanding the stability and selectivity of the protein-ligand complex depends on these interactions. Following molecular docking with the control medicines, the target protein was docked with 28 distinct flavonoids. Due to their potential medicinal qualities, flavonoids, a family of natural chemicals present in many plants, have drawn significant attention in the drug discovery process. In order to find potential binding interactions and gauge their binding energies, the target protein was docked with these flavonoids.
The visualization of the intricate structures and the determination of binding energies were made possible by the molecular docking analysis. These findings can be helpful in directing additional research and medication development efforts because they shed light on the molecular processes behind protein-ligand interactions.

4.4 Docking Analysis

A computational technique called docking analysis is used to examine how a protein interacts with a ligand molecule. In this instance, the investigation concentrated on different flavonoids and compared their binding energies with toremifene while also looking at the interactions between proteins and ligands.

This investigation was conducted to find flavonoids with binding energies comparable to or greater than those of toremifene and tipiracil. The strength of the contact between a ligand

and a protein is measured as binding energy, which is frequently employed as a predictor of a drug's potential effectiveness.

The chosen flavonoids were subjected to Lipinski's Rule of Five. A set of standards known as Lipinski's Rule of Five is applied to gauge a molecule's drug-likeness. To identify whether a molecule is likely to have good oral bioavailability and permeability, it evaluates elements including molecular weight, lipophilicity, and hydrogen bonding. Swiss ADME was used to assess the bioavailability score and bioactivity of the chosen flavonoids in order to further assess their potential. Absorption, distribution, metabolism, and excretion are only a few of the pharmacokinetic and pharmacodynamic aspects of small molecules that Swiss ADME predicts and analyses.

The goal of these investigations was to find flavonoids that not only had high binding energies but also had favourable bioavailability and bioactivity qualities, as well as met the requirements for being drug-like. These results may point to them as prospective candidates for additional therapeutic development and research.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Docking Results for Toremifene and Tipiracil (Control):

The docking outcomes of the receptor molecules NSP 14 and NSP 15 with the traditional medications Toremifene and Tipiracil were examined. Toremifene demonstrated a binding affinity of -7.2, while Tipiracil displayed a marginally higher binding affinity of -7.89. These numbers show how strongly a drug interacts with a receptor molecule. A weaker interaction would be predicted by a lower binding affinity, whereas a stronger interaction would be predicted by a higher binding affinity.

5.2 Docking Results for Flavonoids in accordance with Lipinski's Rule (ADMET Analysis):

Silymarin, Bilobetin, Engeletin and molnupiravir were chosen from the selection of flavonoids as viable candidates because they adhere to Lipinski's rule of 5. Based on their molecular characteristics, substances are evaluated according to Lipinski's rule of five. According to these recommendations, a compound needs to be less than 500 Daltons in molecular weight, have a partition coefficient (log P) under 5, have no more than five hydrogen bond donors, and no more than ten hydrogen bond acceptors. These requirements must be met for flavonoids to be effective as medications that can be taken orally.

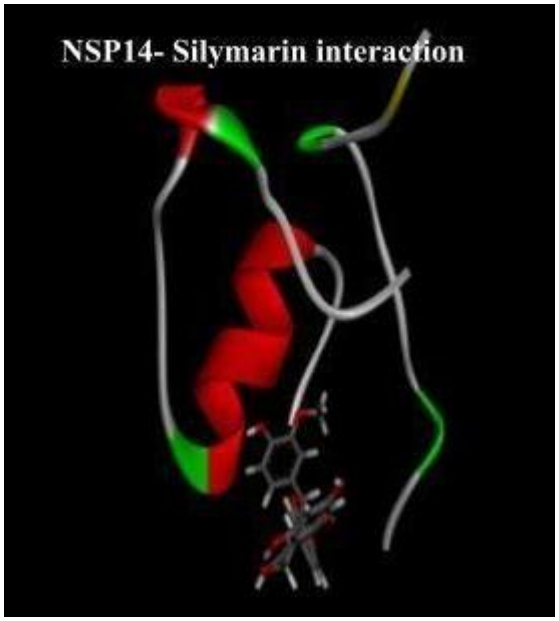

Fig1. Molecular Interactions of Flavonoids Silymarin with NSP 14. (Source- UCSF Chimera)

Fig 2. Molecular Interactions of Flavonoids Bilobetin with NSP15. (Source- UCSF Chimera)

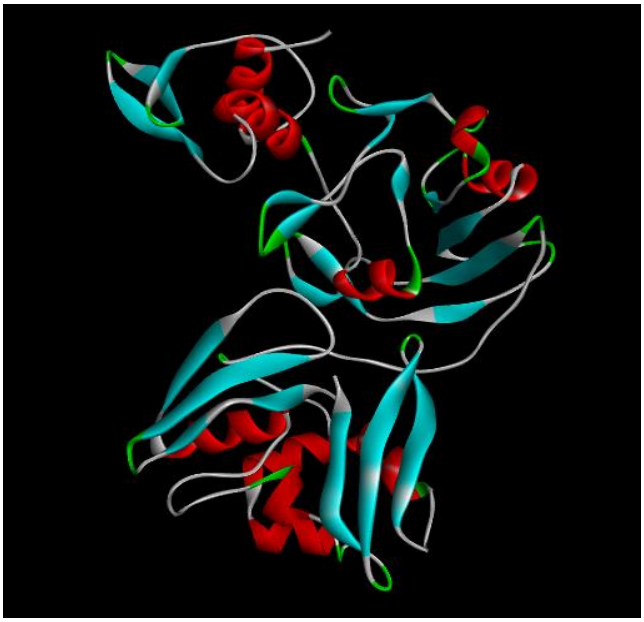

Fig 3. Molecular Interactions of Flavonoids Engeletin with NSP 15. (Source- UCSF Chimera)

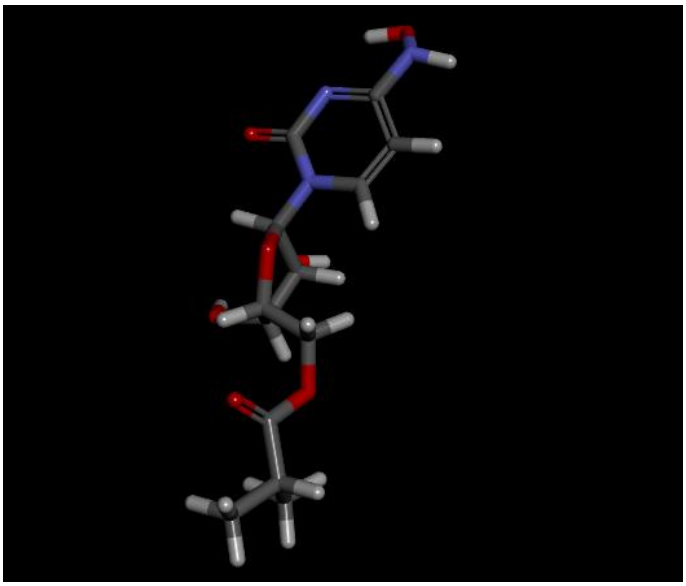

Fig 4. Molecular Interactions of Flavonoids Molnupiravir with NSP 15. (Source- UCSF Chimera)

TABLE 2.IN SILICO PHARMACOKINETICS OF LIGANDS FOR TARGET NSP 15 (USING SWISS DOCK AND SWISS ADME)

Flavonoids	Weight (g/mol)	Hydrogen bond donor	LogP	Hydrogen bond acceptor
Engeletin	434.39 g/mol	6	0.14	10
Bilobetin	552.48 g/mol	5	3.96	10
Molnupiravir	329.31 g/mol	4	-0.89	8

TABLE 3.IN SILICO PHARMACOKINETICS OF LIGANDS FOR TARGET NSP 14 (USING SWISS DOCK AND SWISS ADME)

Flavonoid	Weight (g/mol)	Hydrogen bond donor	LogP	Hydrogen bond acceptor
Engeletin	434.39 g/mol	6	1.77	10
Narcissoside	624.54 g/mol	9	2.62	16
Cosmosiin	432.38 g/mol	6	2.17	10
Isorhoifolin	578.52 g/mol	8	2.98	14

Monoxerutin	654.57 g/mol	10	2.62	17
Silymarin	482.44 g/mol	5	2.79	10
Isovitexin	432.38 g/mol	7	1.94	10

TABLE 4. BIOACTIVITY OF FLAVONOIDS FOR NSP 14 (USING MOLINSPIRATION)

Flavonoid	GPC R ligand	Ion Channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Engeletin	0.1	0.05	0.03	0.11	0.17	0.34
Silymarin	0.07	-0.05	0.01	0.16	0.02	0.23
Isorhoifolin	0.05	-0.32	-0.01	-0.03	0.01	0.24
Monoxerutin	-0.25	-0.9	-0.42	-0.53	0.17	-0.16
Neoeriocitrin	0.07	-0.5	-0.3	0.04	0.07	0.17
Narcissoside	-0.12	-0.66	-0.23	-0.36	-0.13	0.02

TABLE 5 BIOACTIVITY OF FLAVONOIDS (USING MOLINSPIRATION)

Flavonoid	GPCR ligand	Ion Channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Engeletin	0.1	0.05	0.03	0.11	0.17	0.34
Bilobetin	0.04	-0.26	0.13	0.15	0.01	0.04
Molnupiravir	0.64	-0.06	0.19	-0.76	0.28	0.82

5.3 Comparison of Binding:

The binding energies of toremifene and tipiracil were compared to those of the flavonoids. Silymarin's binding affinity, which was -7.45, was equivalent to toremifene's. The binding energy of Tipiracil was surpassed by Bilobetin, which showed a greater binding affinity of -8.03. These findings imply that both Silymarin and Bilobetin interact strongly with receptor molecules and may have significant therapeutic benefits. Bioavailability and Drug Likeness Analysis:

Silymarin and bilobetin not only adhere to Lipinski's rule of five, but also score well in terms of bioactivity and bioavailability. The percentage of a drug's dose that enters the systemic circulation and can be used to produce a pharmacological effect is known as bioavailability. High bioavailability substances are more likely to produce the desired therapeutic effects. The flavonoids may interact with biological targets and cause a pharmacological reaction, according to strong bioactivity ratings.

TABLE 6. COMPARISON OF BINDING ENERGIES OF DIFFERENT FLAVONOIDS AND THEIR RESPECTIVE BIOAVAILABILITY SCORES OF NSP 15. (USING SWISS DOCK AND SWISS ADME)

Flavonoids	**Binding Affinity**	**Bioavailibility Score**
Engelitin	-7.97	0.55
Bilobetin	-8.03	0.55
Molnupiravir	-8.49	0.55
Isorhoifolin	-9.22	0.55
Miquelianin	-9.06	0.55
Ambocein	-10.06	0.55
Diosmin	-9.76	0.55
Isoorientin	-8.26	0.55
Bilobetin	-8.03	0.55
Rutin	-9.04	0.55
Narcissoside	-10.95	0.55
Nepitrin	-8.6	0.55
Molnupiravir	-8.49	0.55

TABLE 7. COMPARISON OF BINDING ENERGIES OF DIFFERENT FLAVONOIDS AND THEIR RESPECTIVE BIOAVAILABILITY SCORES OF NSP14. (USING SWISS DOCK AND SWISS ADME)

Flavonoid	Binding Energy (Kcal/mol)	Bioavailability Score
Engeletin	-7.47	0.55
Narcissoside	-8.37	0.17
Cosmosiin	-7.22	0.55
Isorhoifolin	-7.9	0.17
Monoxerutin	-7.83	0.17
Silymarin	-7.45	0.55
Isovitexin	-6.9	0.55

5.4 Identification of Potential Flavonoids:

Silymarin and Bilobetin have been identified as potential flavonoids for further study based on their adherence to Lipinski's rule of 5, strong binding energies, and favorable bioavailability and bioactivity scores. These substances have the advantageous traits of being ingestible, demonstrating potent interactions with the receptor molecules, and displaying encouraging pharmacological activity. The discovery of these prospective flavonoids offers useful information for the creation of new pharmaceuticals.

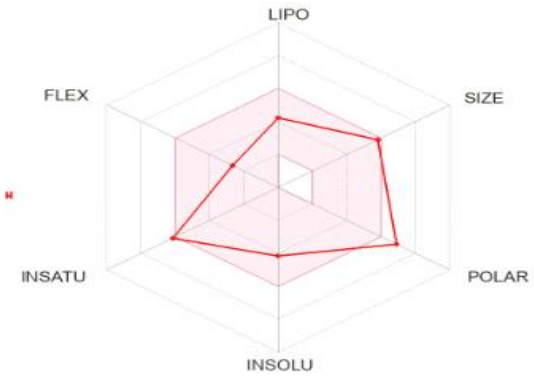

Fig 5. RADAR PLOT OF SILYMARIN

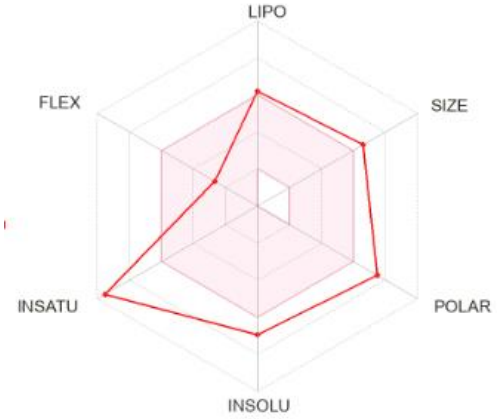

Fig 6. RADAR PLOT OF BILOBETIN

CHAPTER 6

CONCLUSION

The interaction between a protein and a ligand is crucial for developing medications with a structural basis. The SARS-CoV-2 receptors NSP14 and NSP15, two proteins essential for the virus's multiplication and survival, were the focus of this investigation. The goal was to find possible therapeutic agents among flavonoids, natural substances renowned for their various molecular features and health advantages.

The researchers evaluated a variety of flavonoids using computational techniques to compare their binding energies to those of the reference molecules, the medicines toremifene and tipiracil. The stronger the connection between the flavonoid and the viral protein, the higher the binding energy.

It is important to note that additional research is required to confirm the efficacy and safety of flavonoids as therapeutic agents, even though the study offers a positive approach for studying them in this regard. To determine the compounds' antiviral effectiveness, ideal doses, and potential medication interactions, preclinical and clinical investigations are necessary. For optimal absorption and bioavailability, formulation and administration techniques must also be optimised.

In conclusion, this study shows the potential of flavonoids, Silymarin and Bilobetin, as SARS-CoV-2 therapeutic agents. They stand out as attractive candidates for further research due to their greater binding affinities, advantageous pharmacokinetic characteristics, and well-established health benefits. Exploiting the potential of natural substances like flavonoids offers a promising strategy for creating efficient and secure COVID-19 treatments. However, in order to prove their efficacy and ultimately support international

efforts to battle the continuing pandemic, thorough scientific analysis and extensive clinical studies are required.

CHAPTER 7

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in

Second IEEE International Conference on *"Smart Technologies and Systems for Next Generation Computing(ICSTSN 2023)"* held at IFET College of Engineering on 21st and 22nd April 2023

CONFERENCE CHAIR PRINCIPAL CHAIRMAN

DEPARTMENT OF BIOTECHNOLOGY (DTU)

CANDIDATE'S DECLARATION

I, Khyati Rastogi , Roll Number: 2K21/MSCBIO/19, student of M.Sc. Biotechnology, hereby declares that the work which is presented in the Major Project entitled "**INSILICO INTERVENTION TO DELINEATE FLAVONOIDS TARGETING NSP14 AND NSP 15 OF SARS- CoV-2**" in the fulfillment of the requirement for the award of the degree of Master of Science in Biotechnology and submitted to the Department of Biotechnology, DTU, is an authentic record of my own carried out during the period from Jan to May 2023, under the supervision of **Dr. Navneeta Bharadvaja**. I have not applied for any other degree at this or any other University based on the information contained in this report. The following details about the related study have been approved in the IEEE Conference:

Title of the Paper: "Computational analysis of Potential Flavonoids Targeting NSP 14 of SARS-COV-2: A Molecular Docking Approach"

Author Names: Khyati Rastogi, Sanjoli Khare, Navneeta Bharadvaja

Name of Conference: 2023- International conference on "Smart Technologies and Systems for Next Generation Computing" (ICSTSN)– IEEE Conference

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Khyati.

Khyati Rastogi (2K20/MSCBIO/19)

Date : 30/05/23

DEPARTMENT OF BIOTECHNOLOGY

CERTIFICATE

This is to certify that the Project dissertation titled "**IN SILICO INTERVENTION TO DELINEATE FLAVONOIDS TARGETING NSP14 AND NSP 15 OF SARS- CoV-2**" which is submitted by Khyati Rastogi, 2K21/MSCBIO/19, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Sciences, is a record for the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Place: Delhi

Date: 30/05/23

Dr. Navneeta Bhardwaja
SUPERVISIOR

Department of Biotechnology
Delhi Technological University

30/05/2023

Prof. Pravir Kumar

Head of Department
Department of Biotechnology
Delhi Technological University