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From Spice to Science: Computational Insight into *Rhus coriaria* Anti-hMPV Potential

by

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CERTIFICATE OF APPROVAL

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(Iqra Anjum)

Abstract

Human metapneumovirus (hMPV) is considered a key reason of acute respiratory tract infection particularly in infants, elderly individuals and patients with immunocompromised state. Use of natural compounds have gained attention because of their therapeutic effects and biological activity. *Rhus coriaria* is a famous medicinal plant which has been widely used because of its useful bioactive compounds with reported antiviral, antioxidant properties and anti-inflammatory. PubMed literature review was used to enlist the bioactive compounds. Further screening was performed for their medicinal activity using literature. Chemical structure of compound with medicinal activity was retrieved using PubChem. The literature was reviewed to select the target Fusion protein (5WB0) of hMPV for drug target interaction and PDB was used to retrieve the protein structure. Virtual screening was done by using Lipinski's rule of 5 and ADMET properties from pkCSM while molecular docking was performed by PyRx to identify the Lead compound. 30 compounds were selected for computational analysis from *R. coriaria* and Fusion protein (F) (5WB0) of hMPV was selected as target protein. Initially drug likeliness was completed by using Lipinski's rule of 5 to assess oral bioavailability. Among 30 compounds, 23 satisfied Lipinski's criteria and were further evaluated by ADMET analysis. PyRx was used to perform molecular docking and for observing interaction pattern between ligands and target fusion protein of hMPV. Two lead Compounds (Myricetin and Quercetin) were selected after observing the favorable ADMET properties and binding affinities scores of (-7.8 and -7.7 respectively) along with the bonding interactions. For further validation about the stability and dynamic behavior of lead compounds MD simulations were performed at 200ns. The finding of MD simulation has shown strong binding stability and favorable conformational behavior of Myricetin and Quercetin with Fusion protein with acceptable root mean square deviation (RMSD) with, root mean square fluctuation (RMSF) and consistent intermolecular interaction throughout the simulation period. ASP331, GLU294, LYS295, TYR299, ARG329, HIS332, and TYR385 were the most stable connections that persisted throughout the MD simulation of Quercetin. In case of Myricetin Important stabilizing interactions

include hydrophobic contacts with aromatic residues (TYR, PHE) and hydrogen bonds with polar residues (SER, TYR, GLU, ARG). The findings indicates that *R. coriaria* has anti-viral properties and further both *in vitro* and *in vivo* experiments can be performed to verify its beneficial properties and development of novel therapeutics.

Keywords: Human metapneumovirus (hMPV), Fusion protein (F), Rhus coriaria, Sumac, In-silico drug discovery, ADMET analysis, Myricetin, Quercetin and MD simulation.

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Abbreviations

ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
ARIs	Acute respiratory infections
COPD	Chronic obstructive pulmonary disease
hMPV	Human metapneumovirus
hSRV	Human respiratory syncytial virus
LRTIs	Lower respiratory tract infections
MD	Molecular Dynamics
MMMPs	Matrix metalloproteinases
NCBI	National Centre for Biotechnology Information
NLM	National Library of Medicine
PDB	Protein Data Bank
RGD	Arginine-Glycine-Aspartic acid
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation

Chapter 1

Introduction

Acute respiratory infections (ARIs) are considered as main contributor to global mortality causing 4.25 million fatalities impacting countries with lower or moderate incomes in particular [1]. Acute respiratory infections (ARIs) are caused by a variety of viral pathogens. Human metapneumovirus hMPV known as broad spectrum respiratory infection which causes severe lower and mild upper respiratory infections including pneumonia and bronchiolitis. This causes infection in senior population and in individuals who are immune compromised [2] [3]. According to recent research, hMPV can cause major and occasionally deadly consequences in susceptible individuals when paired with RSV and influenza [4] [5].

The first metapneumovirus genus found in humans was classified as part of the Paramyxoviridae family's pneumovirinae subfamily. It is a ssRNA virus with the negative sense inside. Metapneumovirus consists of 8 genes in the RNA genome that encodes 9 different proteins. Another virus called AVM the Avian pneumovirus, also resembles the metapneumovirus genus. This virus shares the similar order of gene as the hMPV [6]. The family Pneumoviridae comprises of two genera: Orthopneumovirus including RSV and Metapneumovirus which includes hMPV [7]. Every age group is susceptible to respiratory tract infections caused by hMPV, although small children and elderly persons with symptoms are more likely to be affected [8].

hMPV can cause non classical and classical infections in respiratory tract including asthma or obstructive pulmonary disease (COPD) symptoms in disease [9] [10]. According to earlier research, hMPV infection is seasonal and usually co-occurs with other respiratory infections [11].

HMPV spreads worldwide and considered as the most common virus causing Acute respiratory infections (ARIs) along with Influenza, Coronaviruses and RSV [12]. With specific routes, hMPV can spread throughout the community and medical facilities. The transmission of hMPV is possible by close contacts such as handshakes, contaminated objects and surfaces, respiratory secretions coming from sneezing and coughing [13]. hMPV outbreaks follow a seasonal pattern which is on its peak in February [14]. Environmental factors are also responsible for transmission of infections including temperature and humidity result in change in viral behavior, host susceptibility, human behavior and environmental conditions [15]. Wang et al. demonstrated a negative correlation between temperature, rainfall levels and the frequency of hMPV cases. This suggests that weather can have an impact on how viruses propagate [11].

Recent studies show the serious effects of respiratory infections delineating about 33 million episodes of RSV are linked with acute lower respiratory infection. Estimated 3.6 million affected children under the age of 5 have been admitted in the hospital in 2019 with approximate death rate of 101,400 [16].

With the staggering 344 million new cases and 2.18 million demises recorded in 2021 worldwide. Lower respiratory tract infections (LRTIs) pose great impact on morbidity and mortality [17]. The growing importance of hMPV in public health discourse has been highlighted by the World Health Organization's recent warnings of outbreaks, notably the 2024 outbreak in China. As hMPV is playing a major role in causing acute respiratory infections ARIs, it is becoming the need of an hour to enhance the understanding about the epidemiological characteristics, spread and clinical features [18]. At present, no specific antivirals are suggested or licensed for clinical use [19]. The preventive measures including oxygen supplementation and intravenous hydration are in use for the hospitalized children and infants.

Corticosteroids and bronchodilators are being used empirically but no data is available for confirming its efficacy [20] .

In children under five, acute respiratory infections (ARIs) are the second most common cause of mortality globally. Approximately 5-7% of pediatric pneumonia hospitalizations are linked to human metapneumovirus (hMPV) [21].

According to several publications, hMPV positive ranges from 8.5 to 11% in Argentina and Canada, respectively. However, earlier research from Pakistan revealed a comparatively low frequency of 5.2% and 7% [22].

A nucleoside analogue known as Ribavirin has been explored for the treatment of hMPV. Ribavirin functions by inhibiting the viral RNA polymerase and disrupting viral purine metabolism. This also demonstrates immunomodulatory effects through up-regulation of CD4 and CD8 T lymphocyte-derived cytokines and down-regulation of Th2 cytokines like IL-10 [23]. Although there is a conflicting data about its therapeutic benefits [24].

There are different routes for administration presenting a unique challenge: aerosolized administration is associated with high costs, oral delivery may face poor bioavailability, teratogenic risks for health care workers and possible decline in respiratory functions, while intravenous administration yields inconsistent and limited results. Distinguished success has been revealed after combining IVIG and Ribavirin in immunocompromised patients specifically those with hematological conditions and lung transplant recipients [20] [25].

Medicinal plants are in use against viral infections while modern research is confirming the antiviral properties of plants as well. Many plants including *Echinacea purpurea* which can boost the immune system by inhibiting the viral entry and replication of herpes and influenza virus [26]. *Glycyrrhizin* is present in *Glycyrrhiza glabra* (licorice) having the ability to show efficacy against hepatitis C and SARS-CoV by disrupting the proteins of viral envelope [27]. *Zingiber officinale* (ginger) contains gingerols which can restrict the viral attachment in respiratory syncytial virus [28]. These plants have been proven as natural antiviral agents.

R. coriaria also known as Sicilian or tanning sumac, belongs to Mediterranean region. This is a deciduous shrub valued for its tart, red drupes which are used as traditional medicine and spice. Technical synonyms include Rhus, sumac (L.) and its variations, such as *Toxicodendron vernicifluum* (Stokes) F.A. Barkley (previously *R. verniciflua*), an East Asian lacquer tree with comparable bioactive profiles that needs to be processed without the use of toxins because of its Urushiol level. *R. coriaria* has shown antidiabetic, anti-inflammatory, antioxidant, antimicrobial, and hypolipidemic effects because of presence of high level of polyphenol (e.g., gallotannins, flavonoids like quercetin and myricetin) [29].

While the organic acids along with tannins help in healing wounds, cardiovascular protection, gastrointestinal disorders (e.g., diarrhea, ulcers) and liver health by producing free radicals and modulating enzymes including xanthine oxidase. Antivirally, extracts inhibit enveloped viruses like HIV-1 (reverse transcriptase inhibition), influenza, varicella-zoster, and hepatitis B (reduction of HBsAg secretion), Herpes simplex types 1 and 2 (through the root bark of *R. aromatica*, IC50 $\sim$20-50 μg/mL), and tobacco mosaic virus (up to 80% inhibition) [30].

T. vernicifluum exhibits broad-spectrum activity against respiratory viruses, possibly through flavonoid disruption of viral envelopes. Many computation studies suggested that Hinokiflavone and Myricetin have the capability to bind SARS-CoV-2 main protease efficiently, supporting adjunctive roles in COVID-19 prophylaxis [31].

R. coriaria is enriched in multiple polyphenols (gallotannins, flavonoids (e.g., quercetin, myricetin) and some organic acid that possess antiviral properties against hMPV which cause respiratory disease. Researches have revealed that *R. coriaria* can inhibit the activity of herpes and influenza, so the bioactive compounds may also have same effect on disrupting viral envelope or prevent hMPV from adhering to the host cell.

This study uses computational methods to investigate the antiviral potential of *Rhus coriaria* (sumac) against human metapneumovirus (hMPV). Its scope is to find bioactive substances and predict how they will interact molecularly with viral

target protein. These results could serve as a basis for experimental confirmation and the creation of new antivirals. Molecular docking studies can be applied to investigate the contact between hMPV fusion proteins and flavonoids to explore the use of Sumac efficacy against the related respiratory viruses [30, 31].

1.1 Problem Statement

The absence of antiviral treatments for Human Metapneumovirus (hMPV) necessitates the investigation of therapeutics including natural inhibitors. While *R. coriaria* contains bioactive compounds with known antiviral activity against enveloped viruses, its efficacy against hMPV and the molecular basis for any potential inhibition are entirely unknown. There is a need to address this gap to identify potential lead compounds as an inhibitor against hMPV.

1.2 Aim of Study

The aim of this study is to computationally evaluate the potential of bioactive compounds from *Rhus coriaria* to act as inhibitors of Human Metapneumovirus (hMPV) by determining their binding interactions with target viral proteins.

1.3 Objectives

i. To identify and retrieve the key bioactive compounds from *Rhus coriaria* from phytochemical database.

ii. To retrieve and prepare the 3D structure of hMPV protein, the Fusion (F0) glycoprotein.

iii. To priorities the lead compound using Lipinski's rule of 5, ADMET properties and molecular docking.

iv. To perform Molecular Dynamic simulation to assess the structure stability of protein ligand complex.

Chapter 2

Literature Review

2.1 Human Metapneumovirus

The main reason of respiratory tract infections, which are more common among young children, elderly individuals, and those having compromised immune systems, is human metapneumovirus (hMPV). Human metapneumovirus is abbreviated as hMPV that was discovered in 2001 in Netherland [32]. hMPV is a virus which has single stranded RNA with a genome of 13.3 kb length composed of 8 genes with 9 proteins [33] Patients of hMPV are diagnosed with pneumonia, bronchitis and bronchiolitis. Most common symptoms in them are cough, fever, hypoxia upper and lower respiratory tract infection and wheezing [34] While major cause for hospitalization of patients are pneumonia and bronchiolitis [35].

2.1.1 Taxonomy of hMPV

The virus was isolated from pediatric patients having symptoms similar to the infection of hSRV. However, it has been moving around in the human population for almost 60 years [32]. hMPV belongs to the family Pneumoviridae within the order Mononegavirales [36]. hMPV formerly categorized as a subfamily win the Paramyxoviridae.

Pneumo- and paramyxoviruses' large (L) proteins differ phylogenetically, and the presence of a conserved M2 gene caused them to be reclassified as a distinct viral family in 2016 after they had previously been categorized as a subfamily under the family Paramyxoviruses [37].

Based on differences in their genome organization and related sequences, the Pneumoviridae family is divided into two distinct genera, Orthopneumovirus and Metapneumovirus. In genome of Orthopneumovirus ten genes are present, exceeding the actual genome length of metapneumovirus having 8 genes in it.

Viral species that are present in Orthopneumovirus can reason breathing infection in humans, rodents and cattle. However, in metapneumovirus human and avian pneumoviruses are included. hMPV is a member of the metapneumovirus family, which is further classified into various genotypes depending on the glycoprotein which provides the attachment site.

There are two distinct genotypes of hMPVs (A and B). These genotypes are subdivided into four subtypes A1 and A2 is subdivided into A2a, A2b and A2c lineages.

While B1 and B2 is categorized into B2a and B2b [38] [39] [40]. These duplications have no clinical relevance found so far. hMPV and hRSV human respiratory syncytial virus which are closely linked.

Influenza viruses (IVs) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are the main causes of respiratory tract infections in pediatric patients with comparable seasonal epidemiology and overlapping clinical symptoms, with late winter and spring peaks [41] [42] [43].

Human infections belonging to the Paramyxoviridae family, such as measles virus (MeV), mumps virus (MuV), and human parainfluenza viruses (hPIVs), are distantly related to hMPV.

The inclusion of an M2 gene and differences in L genes distinguish pneumo- and paramyxoviruses from one another, although their viral entrance and replication mechanisms are similar.

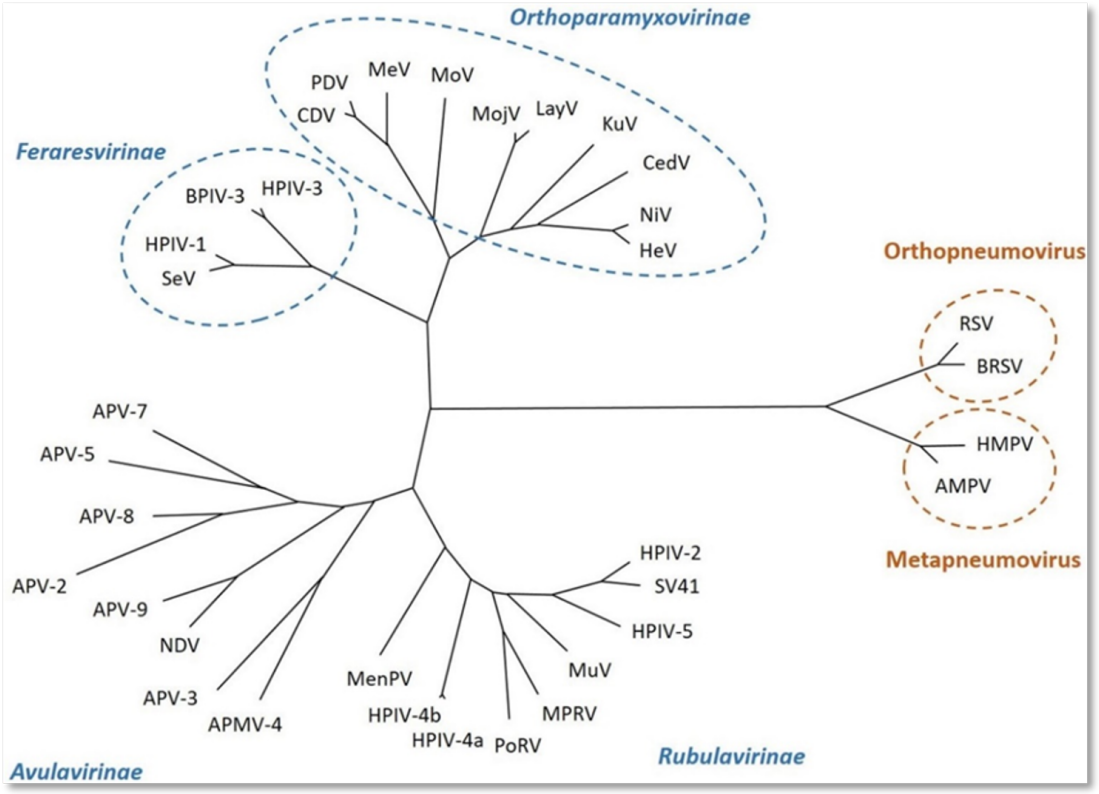


FIGURE 2.1: Unrooted phylogenetic tree of Pneumoviridae family members (Orthopneumovirus and Metapneumovirus in orange) and Paramyxoviridae (blue) based on the protein sequence [44].

2.1.2 Epidemiology

HMPV was first discovered by a Dutch researcher in 2001 and epidemiology has been reported in Italy, Canada, France, Spain, Japan, Australia, and Germany. Moreover, virus was also found in South African children who were HIV positive with no weak immune system [45]. Furthermore, the virus's isolation within Europe and Canada throughout the last ten to twenty years, along with the Netherlands' 1958 immunological proof of human contamination, have shown researchers believe hMPV is not a novel agent. Even though hMPV infection is frequent among children, instances of extreme breakouts in elderly and re-infection in people with weak immunity show that novel infections may happen during life as a result of either acquiring new genotypes or immune responses that aren't totally protective [46].

2.1.3 Structure and Viral Genes

hMPV is a virus which has single stranded RNA with a genome of 13.3 kb length composed of 8 genes with 9 proteins. Figure 2.2 shows genomic sequence of hMPV is 30'-N-P-M-F-M2-SH-G-L-50' in which each gene is encoded by single protein except M2 gene.

M2 encodes for two different proteins (M2-1 and M2-2) because of presence of two ORFs in its mRNA. Glycoproteins are present on the surface which are linked to M matrix protein, helpful for the attachment to the inner membrane [33].

There are three glycoproteins which are present in the surface of hMPV including the attachment protein (G), small hydrophobic protein (SH) and fusion protein (F). The SH protein is called as small hydrophobic protein that decreases transcription in gene.

It is a type II transmembrane protein that can use the transcription gene path and participate in NF-κB transcriptional activity, the small hydrophobic (SH) protein reduces gene transcription [47], [48], [49].

SH protein plays vital part in life cycle of virus by altering permeabilities of membranes [48]. Another protein called G protein is found in cell membrane that encourages the attachment of viruses causing hMPV infections. G protein is a glycosylated protein with many of O-and N- linked sugars in it [50].

Fusion protein (F) is produced as an inactive precursor (F0) in the virus. There are two subunits F1 and F2 joined by disulphide bonds which must be cleaved in order to biologically active protein. Upon the combination of hMPV RNA with the putative transcription factor (M2-1), large polymerase protein (L), nucleoprotein (N), and RNA synthesis regulatory factor (M2-2), the nucleocapsid is assembled [33].

The viral genome is encapsulated by nucleoprotein N that shield it from nucleases. The ribonucleoprotein (RNP) complex can only be gathered because of interaction between the P and N proteins [51] [52].

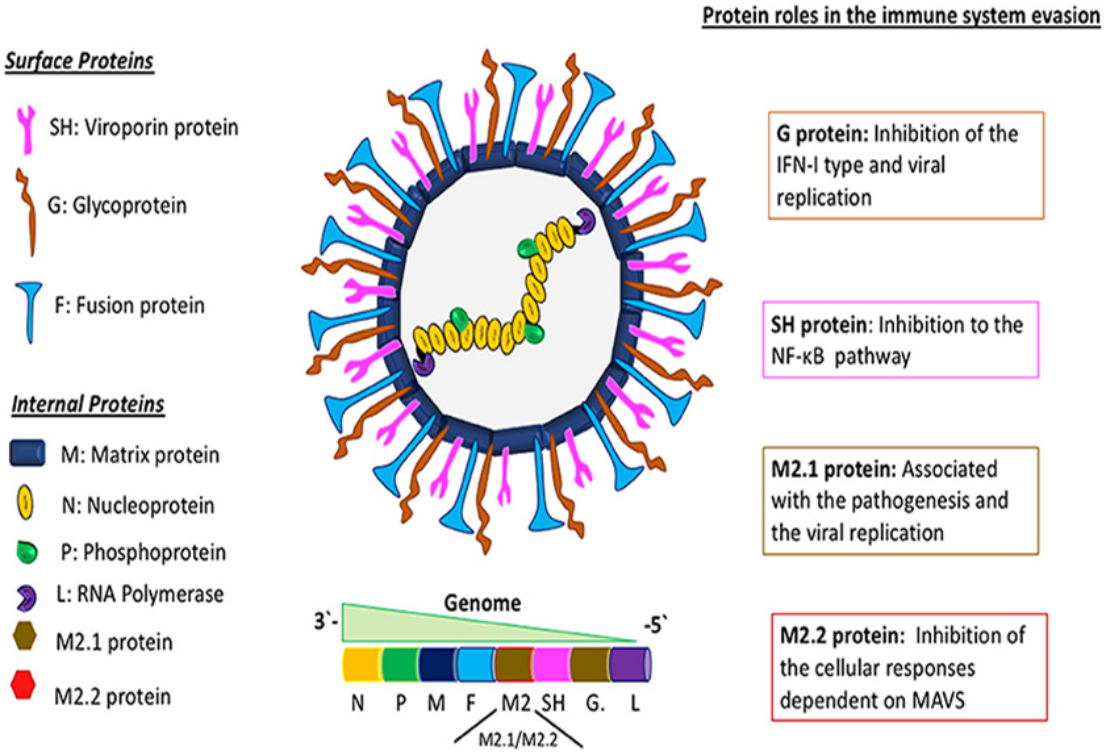


FIGURE 2.2: Structure and viral genes sequence of hMPV [53].

2.1.4 Clinical Features of hMPV

The symptoms of hMPV and hSRV are not easily distinguishable particularly in young children. People with hMPV tend to have bronchiolitis, pneumonia, and bronchitis. Most common indications in them are cough, fever, wheezing, hypoxia upper and lower respiratory tract (LRT) infection [34]. While major cause for hospitalization of patients are pneumonia and bronchiolitis [35]. Durations of fever on average is about 10 days in hMPV positive cases with a peak during illness course [41].

In young adults who are reinfected with hMPV they will show symptoms like mild cold and flu along with fever while on the other hand if elderly patients are reinfected, they show severe symptoms like pneumonia or even death [54]. A study reported that 50% of hMPV patients who are children were diagnosed with otitis media [55]. One more study has reported hMPV infected 8% of children who visited hospital wheezing [56]. Both children and adults with hMPV have been diagnosed with exacerbations of their asthma [57].

RT-PCR was used to detect hMPV in asymptomatic children. However, their viral loads were far lower than those of children who experienced symptoms [58]. Regardless of genotype, greater hMPV virus loads were substantially connected with severity of the sickness and the duration of illness [59]. Lower peripheral blood lymphocytes, a higher monocyte ratio, and a minor to moderate rise in C-reactive protein (CRP) levels were characteristics of the initial phases of hMPV infection. As the mitigation of symptoms progresses it results in the normalization of peripheral blood lymphocytes and ratio of monocytes, while the CRP levels continued for some time [41].

hMPV infected children with elevated serum CRP levels are also reported to have leukocytosis and leukopenia [60]. For one to two weeks following severe sickness, there was a high incidence of hMPV viral shedding [61] [62]. Lung transplant recipients may experience a variety of infections as a result of hMPV [63].

2.1.5 Pathogenesis

Persistent hMPV infection may result from a weak and delayed immune response, along with postponed cytotoxic T-cell action that hinders effective elimination of the virus after the initial infection [64]. hMPV inhibits superantigen-driven T-cell activation by infecting dendritic cells, which restricts the development of antigen-specific CD4+ T cells and influences the establishment of long-term immunity [65]. In contrast to RSV and influenza, hMPV causes significantly lower amounts of cytokines such IL-12, TNF-α, IL-6, IL-1β, IL-8, and IL-10. Respiratory viruses frequently modify cytokine responses [66].

In cotton rats and BALB/c mice, hMPV infection results in pulmonary inflammation, which results in increased levels of interleukins (IL-2, IL-8, IL-4), interferon-α (IFN-α), macrophage inflammatory protein-1α, and monocyte chemotactic proteins in lung tissue and bronchoalveolar lavage fluid. Perivascular and peribronchiolar [67].

Immunological and histological analyses show signs of hyaline membrane disease, smudge cells, intra-alveolar foamy, damage in alveolar sites and haemosiderin-laden macrophages [63]. Toll-like receptor (TLR)-dependent signaling is known to be activated by hMPV infection; however, the precise function of TLR-mediated pathways in host defense and disease development throughout pulmonary hMPV infection is yet unidentified.

Notably, a recent study found that after intranasal hMPV infection, MyD88-deficient mice display considerably less lung inflammation and disease severity than wild-type C57BL/6 mice [68].

Figure 2.3 summarizes the molecular processes that underlie hMPV pathogenesis. Whether hMPV infection is restricted to the respiratory system or if the virus can spread throughout the body is still unknown. A little amount of data points to a potential systemic hMPV involvement in middle ear fluid in one study, and RNA of hMPV was found in the brain tissue of a patient who passed away from encephalitis in another. Nevertheless, further investigation is required to validate and elucidate these results [69].

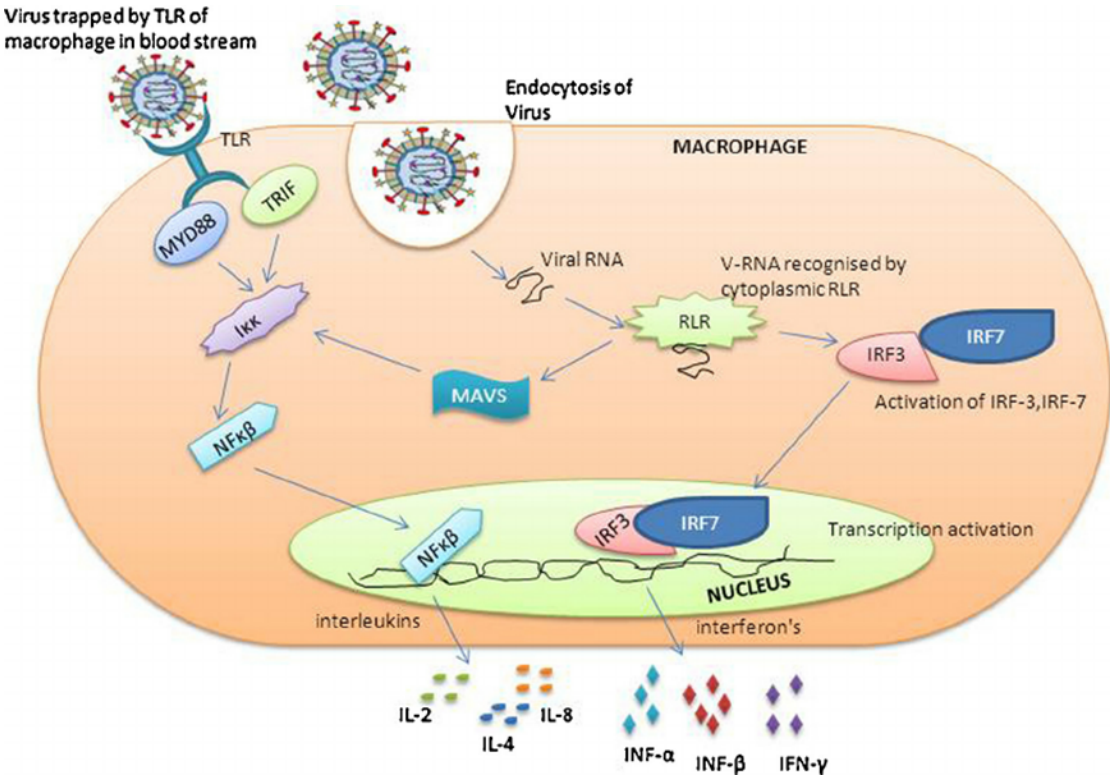


FIGURE 2.3: Pathogenesis of hMPV infection [52].

2.1.6 Treatment and Control Strategies

hMPV is transmitted through contaminated surfaces and respiratory secretions. Spread by these means can be limited by maintaining good hygiene and counter measures. No proper treatments are licensed for these viral infections although ribavirin has been in use in severe cases. There is no vaccine developed for hMPV and hRSV but many other approaches are in testing phase. Presently, palliative care is suggested to recover from the inflammation in the respiratory tract from infection. Therapy through oxygen, corticosteroids and mechanical ventilation have been in use against symptomatic treatments. Moreover, as monoclonal antibody therapies are used against HRSV infection so it is also possible to use them against hMPV. Although this method is in developmental stages so it needs more research to confirm the results. Treatment through monoclonal antibodies has shown promising results in hamster, coon rat models and mouse, but it has not been in use for humans yet [70].

Currently, supportive care is the main therapy option for hMPV infection. Few reports have suggested the use of ribavirin, fusion inhibitors, minor interfering ribonucleic acids and immunoglobulins for control and treatment purpose of hMPV infection [71]. Many vaccines have been tried and tested in non-human primate and rodent models. The testing of vaccines has shown positive results but none of this vaccine is tested against human volunteers. Heat inactivated viral vaccine when used against hMPV has seen to enhance the effect of lungs disease in mice [72].

T cells epitope vaccines have reduced the immune modulation by hMPV challenge. After being challenged with hMPV, mice who received a hMPV cytotoxic T cell epitope vaccination created less Th1 and Th2 type cytokines than mice that were not vaccinated [73]. Several other studies are also performed for evaluation of hMPV infections with the help of immunization by chimeric vaccines. When these vaccines were experimented in African Green Monkeys and hamsters showed immunity against a challenge with the wild type by neutralizing the antibodies [74].

A subunit vaccine by using Fusion protein (F) from hMPV has shown to make cross protective immunity against hMPV in hamster [75]. Many hMPV F subunit vaccines have shown very strong level of defense after testing for hamster, rodents and non-human primates. Recently, hMPV virus like particles VLPs having mimicking properties like viral surfaces of subgroup A and B tested as vaccine. Results shown an induction of a powerful humoral immunological response against heterologous and homologous strains [76]. However, more research is needed to develop a vaccine that will protect against all hMPV subgroups, even if the hMPV-VLP vaccine seems to be an effective approach.

Reverse genetics techniques based on plasmids has greatly accelerated efforts to create a live vaccination that protects against hMPV infection [77]. It has been shown that the deletion of the SH, G, or M2-2 genes has no impact on the immunogenicity or antigenicity of the virus whenever recombinant hMPVs with these gene deletions are tested for virus replication levels [78].

Another live, weakened hMPV vaccine strain was formed in new research by changing the F protein's glycosylation site. Despite of challenge for 56 days after inoculation, this vaccine was about to provide full defense [79]. All of these results imply that a more thorough understanding of the molecular pathophysiology of hMPV is necessary before an effective vaccination against it can be created.

2.1.7 hMPV's Viral Structure and Replication

hMPV is enclosed in a pleomorphic shape having 150-600nm diameter. Virus is single stranded, non-segmented RNA genome consist of 8 genes encoding for 9 proteins from 3 to 5. Nucleoprotein (N), phosphoprotein (P), matrix proteins (M), fusion protein (F), small hydrophobic protein (SH), glycoprotein (G), and large protein (L). M2 (encoding two overlapping proteins: putative transcription factor M2-1 and RNA synthesis regulatory factor M2-2) [80]. Non-coding areas surround each open reading frame (ORF), aiding in the control of transcription termination and re-initiation. Gradient mRNA consequently diminishes from the 3' to the 5' end of the genome [81].

The avian metapneumovirus (aMPV), which relates to the Metapneumoviridae family and genus Metapneumovirus, has a genetic makeup that is identical to that of hMPV [82]. During replication, the P protein stabilizes the viral polymerase L to ensure the ribonucleoprotein (RNP) formation. M2-1 and M2-2 aids in regulation of the polymerase of virus and modifies response by immune system of host [83].

M2-1, M2-2 N, P and L are associated with RNA genome and forms nucleocapsid. The M protein that envelops the lining of the inner membrane of the lipid bilayer envelope encloses the nucleocapsid. M protein is essential for viral budding and assembly [84]. Furthermore, This protein also helps in the interaction between nucleocapsid and viral envelope also it will stop the activity of transcriptase before nucleocapsid packaging [85]. It is also assumed that cellular immune response is modulated by M protein by stimulation of formation of inflammatory cytokines [86].

Viral envelope of hMPV consists of 3 glycoproteins and SH, F and G. F protein is the determinant of major antigenic structure needed for viral entry into the receptors cells including Arginylglycylaspartic acid (RGD)-binding integrins and Glycosaminoglycans. This will initiate the fusion of virus into the host cell membrane to release the viral genome into the cytoplasm [87]. The Glycolinteractome is linked to the Fusion protein, its attachment and entrance processes, as described in detail in recent research [88].

N-linked and O-linked glycans that are mostly present on the human lung epithelium bear a striking resemblance to these bound patterns. Lacto-N-neotetraose is one such glycan that may help prevent hMPV infection by disrupting the cellular receptors that enable entrance of hMPV. It is also a major target for antibodies that neutralize it [75].

The F protein of paramyxoviruses and hMPV along with other pneumoviruses share functional and structural similarities [89]. Fusion protein of hMPV is type 1 glycoprotein working as a trimmer on the surfaces of virus. It is produced as precursor protein F0, which is inactive in nature. This F protein will be divided by extracellular host cell proteases to become active protein [90]. After cleavage of

F0 protein it consists of F1 and F2 which is linked by disulfide bond. Apart from facilitating fusion between the membranes of the virus and the host cell, hMPV F may also promote fusion between adjacent cells in vitro [45].

G proteins of pneumovirus functions differently than the glycoproteins paramyxoviruses. G proteins mediate the attachment of hMPV to the host cells membranes by binding to glycosaminoglycans but it does not play any role in viral replication and infection. Viruses which are modified and lacks G proteins are still able to infect and reproduce in hosts [78]. Along with mediating attachment, G protein has ability to modulate the immune response. SH protein disrupts the innate defense via altering hMPV fusogenicity and inhibiting nuclear factor [48].

The initial stage for replication is virus attachment to the host cell via binding G and F protein to cellular receptors in Fig 2.4. Similar to other viruses, hMPV reaches host cells by either endocytosis after fusing with the endosomal membrane or by fusing with the plasma membrane [91].

The RNP of the virus contains negative sense viral RNA (vRNA) is going to be released into the cytoplasm following the fusion of the membranes of the virus and the cell. The viral polymerase complex utilizes RNPs as templates to synthesize RNA and synthesize full-length antigenomic cRNA. The full-length vRNA will be produced using cRNA as a template. This VRNA might be used for secondary transcription or incorporated into new virions. At the 3 and 5 ends of the vRNA, leader and trailer sequences serve as promoters, guiding the transcription of mRNA and the synthesis of cRNA. The M2-2 protein of hMPV is thought to regulate RNA production by switching the scales from mRNA to vRNA synthesis, similar to hRSV [92] [93].

Pneumoviruses' viral polymerase complex is made up of L monomers and P tetramers. Three enzyme domains compose this structure. RNA-dependent RNA polymerase (RdRp) is the first, second is PRNTase, or polyribonucleotidyltransferase Methyltransferase (MTase) is a third enzyme.[94] [95]. RdRp aids in the transcription of vRNA into polyadenylated mRNAs and the replication of the viral genome. PRNTase and MTase are in control of mRNA capping and methylation,

respectively. The newly synthesized RNPs and M proteins travel straight to the plasma membrane. On contrary, surface proteins F, G, and SH mature after passing through the endoplasmic reticulum and Golgi apparatus. After assembly, new virions releases from the plasma membrane.

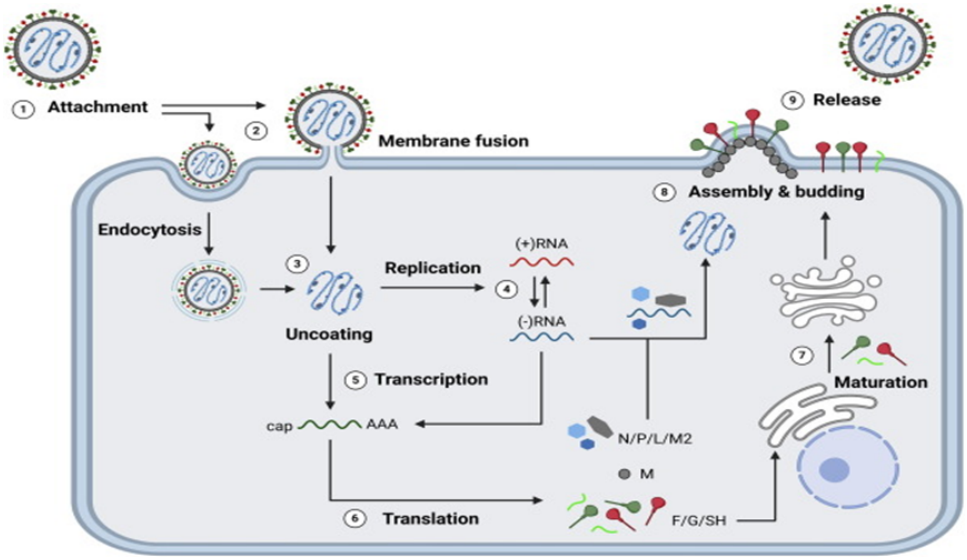


FIGURE 2.4: The viral replication cycle of hMPV [44].

2.1.8 Fusion Protein

Integrins may be hMPV F's functional receptors since the hMPV F protein has a RGD motif that is significantly conserved that is typical of protein that bind integrins. However, additional research revealed that hMPV F interacts with integrins following an initial binding to proteoglycans and integrins may raise the infectivity of hMPV by a mechanism that is yet unclear [87] [96].

F glycoprotein is produced as an inactive precursor F0 which needs proteolytic action to develop protein that is suitable for fusion. The precursor F0is sliced only once by proteases which is trypsin like outside the cell. Generation of cleavage will make two subunits F1 and F2 which remain covalently linked by disulfide bond. The matured hMPV F protein forms a trimer composed of disulfide-linked heterodimers and is combined into viral particles in a metastable pre-fusion state. Throughout the membrane fusion process, this glycoprotein experiences a sequence of temporary conformational changes before adopting a highly stable post-fusion

structure. In some viral strains, exposure to acidic environments enhances hMPV F-mediated membrane fusion. Although low pH does not appear to be a universal trigger for hMPV F activation, investigations into pH-dependent fusion have highlighted specific regions of the protein that are likely important for the conformational transitions involved in membrane fusion [97].

2.2 An Overview of Using Therapeutic Plants to Treat Viral Diseases

Therapeutic uses of plants against diversity of viral infections from ancient times till date. Among the oldest culture the most common is ayurveda of India, *Eber papyrus* of ancient Egypt and traditional Chinese medicine are practicing medicinal plant for treating infections and documented manuscript are also available [98] [99] [100]. Chinese are using traditional medicine for more than 3000 years. The earliest medical literature of any civilization is based on that approach and explains how plants can be used medicinally. [101]. Common examples is Ephedra (*Ephedra sinica*) used in China for the treatment of common cold [102], Green tea (*Camellia sinensis*) has been in use against hepatitis C and B, herpes and Epstein-Barr viruses [102] [103] [104].

Details of plant and natural treatments against a wide range of illnesses, as well as different viral infections, may be found in the Egyptian papyrus [105]. Most common plant is Allium sativum (garlic) which is used against influenza, recurring colds and respiratory catarrh [106]. Echinacea purpurea also known as Echinacea, also reported efficient against respiratory viral infections [107]. According to the three-thousand-year-old Indian natural medicine system known as Ayurveda, herbs comprising Azadirachta indica, Ficus religiosa, and Aegle marmelos are effective against a range of viruses [98] [108] [109]. Plants have also long been used in traditional African medicine and European herbal medicine to treat a variety of ailments, as well as viral infections. For example, Elderberry (Sambucus nigra) has long been used as a cold and flu cure throughout Europe [110]. While African

native herbs like Artemisia afra and Sutherlandia frutescens have been shown to be effective against a variety of viral illnesses. The use of remedial plants as an substitute treatment for a variety of viral diseases has increased throughout the past several decades [111]. This is partly because viral infections are becoming more common and antibiotic resistance is becoming a bigger concern [112].

2.2.1 Mode of Action of Secondary Metabolites from Remedial Plants as Antiviral Agent

Secondary metabolic products are organic compounds that are produced by plants and play a role in their growth, development, and reproduction. These metabolites are used by plants to survive against the adverse conditions of surrounding environment along with carrying important physiological tasks [113]. Chemical structure, constituent element composition, and solubility in organic solvents or water are some of the characteristics used to categorize secondary metabolites in plants. Although the biosynthetic pathway is one of the widely used and well-known criteria [114]. On the basis of these pathways 3 classes of secondary metabolites are known in medicinal plants 1) Phenolics 2) Alkaloids 3) Terpenoids. Aforementioned compounds show pharmacological effects against various viral agents [115].

Most common mechanism of action of secondary metabolites derived from plant against viruses include are as follow:

i. Entry of virus through attachment.

ii. Inhibitory action of viral replication.

iii. Inhibition of protein synthesis.

iv. Modulation of hosts immune system.

v. Disruption of signaling pathways within cells.

vi. A strong virucidal effect [116].

About 20 years after metabolomics first became popular, certain improvements were made in the study and production of natural products and therapeutic plants. A great possibility of research is here for investigation of metabolomics in medicinal plants along with developing new therapeutic agents [117].

2.2.2 Phytochemical and Remedy for hMPV

Many useful chemical compounds are formed by plants to carry many metabolic activities. Secondary metabolites produced by plants are used in various pharmaceutical companies, fragrance, food additives and colors etc.[118]. Many parts of plants or plants as whole are useful against hMPV virus. Several active phytochemicals including flavonoids, terpenoids, vanillic acid, lignans, apigenin, saponin, tannin and isovanillic acid have revealed useful effects against the viral replication, membrane permeability and cellular processes [118] [119] [120].

Plants produce various kinds of flavonoids including flavonols, isoflavanoids and anthocyanidins. These compounds are responsible for showing antiviral effects. Furthermore, quercetin also inhibits the activity of NF-κB that is responsible for expressing genes in hMPV [120]. Different plants including *Litchi chinensis*, *Camellia sinensis* and *Phragmanthera capitata* own antiviral activities [121]. *Prunus dulcis* has the ability of blocking of viral bindings in vero cells [120]. *Azardirachta indica* (Neem) and *Cassia fistula* (Golden Shower) both plants are used for antiviral properties because of the existence of metabolites, such as Alkaloids, flavonoids, Kaempferol, and a proanthocyanidin [119] [122]. Polyphenols are among the plant antioxidants that can reduce oxidative stress and shield cells from viral harm. Quercetin, an antioxidant that strengthens the immune system, is found in Aloe vera (L.) and *Allium sativum* L. (Onions) [123] [124].

2.3 *R. coriaria* Habitat and Distribution

The scientific name for Sumac is Rhus coriaria Mediterranean plant, which is a member of the Anacardiaceae family. It is used as natural spice and as flooring

agent in many countries [125]. *R. coriaria* is grown and originated in temperate and tropical regions in world. The name came from word "Summaq" which means dark red in Arabic and Syriac term [126].

2.4 Morphological Features of *R. coriaria*

R. coriaria grows into a shrub which stands three to four feet tall and bears pinnate leaves that cluster in pairs of between six and eight tiny leaflets. It has bunch of white flowers at its terminal. Fruit of this plant is spherical in shape which becomes reddish when ripped [126] (Figure 2.5). The fruit of plant is villose and reddish drupes after ripening with one seed. They consists tannins, various organic acid, phenolics, flavonoids, sugar and many essential oils etc. [127].

FIGURE 2.5: (*R. coriaria* plant Fruit, Leaves and Powder) [128].

2.4.1 Taxonomic Classification of *Rhus coriaria*

The scientific name for Sumac is *Rhus coriaria* a Mediterranean plant, which is a member of the Anacardiaceae family. Taxonomic classification is given in table 2.1 below.

TABLE 2.1: Taxonomic Classification of *Rhus coriaria* [128].

Kingdom	Plantae
Sub kingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Subclass	Rosidae
Order	Sapindales

Table 2.1 continued from previous page

Family	Anacardiaceae
Genus	Rhus
Species	*Rhus coriaria*

2.4.1.1 Vernacular Names

Sumac is grown and originated in temperate and tropical regions in world. Name Sumac came from word "Summaq" which means dark red in Arabic and Syriac [126]. Some of the most common vernacular names are mentioned in table 2.2 below.

TABLE 2.2: Vernacular names of *R. coriaria* worldwide [129].

Sr no.	Language	Common Name
01	Persian	Samaka, Samak, Sumaq
02	Hindi	Tatrak, Tatri
03	Arabic	Timtima, Tamtam, Sumak, Sumac
04	Urdu	Sumaq
05	English	Sumach, Sumak
06	Bengali	Sumok
07	Kashmiri	Samak

2.4.2 Physical Characteristics of *R. coriaria*

R. coriaria is a shrub that reaches a height of three to four feet. Its pinnate leaves form groups in pairs of six to eight tiny leaflets. It has white flowers at its terminal. Fruit of this plant is spherical in shape which becomes reddish when ripped [126]. Some of the important physical properties of *R. coriaria* are mentioned in table 2.3 below.

TABLE 2.3: Physical Properties of *R. coriaria* fruit [130].

Sr no	Physical properties	Measurement
01	Length	4.70 mm

Table 2.3 continued from previous page

Sr no	Physical properties	Measurement
02	Weight	0.20 g
03	Volume	19.50 mm^3
04	Geometric diameter	3.64 mm),
05	Sphericity	0.77
06	Thickness	2.64 mm
07	Moisture content	4.79 %
08	Porosity	68.50%)
09	Static friction	0.48-0.68
10	Bulk projected area	0.16 cm^3
11	Terminal velocity	3.50 m/s
12	Density	304.25 kg/m^3

2.4.3 Ecology and Cultivation

R. coriaria reproduced by the help of seeds which are (spread by birds and animals), shoots from rhizomes forming large colonies. Researches claim that plant grows in mountainous areas. This is a wild plant, grown in tropical regions and on riverbanks. Plants can be cultivated on eroded soil and have short roots to decrease soil drift. It has been grown in Mediterranean and Middle East where it is also used in tanning industries. It is also found in Afghanistan, Canary Islands as it is commonly present in Turkish and Mediterranean territories [131] [132].

2.4.4 General Composition of Vitamins and Minerals Contents in *R. coriaria*

he overall composition of the *R. coriaria* fruit in dried form contains: water-soluble extract (63.8%) moisture (6-11.8%), ash (1.5-2.66%), essential oil content (1.0%) fatty oil (17.4%) fiber (14.6-22.15%) and protein (2.3-2.6%). (Kizil and Turk., 2010). The mineral composition of *R. coriaria* fruits has (K, Ca, Mg, P, Fe, Na, Zn, Mn, Cu, and Al) as dominated elements. This mineral composition was found

by plasmic atomic spectrometer [133], [134], [135]. Mineral content is dependent on the geographical location and environmental factors where the plant is grown. On the other hand, plant is enriched with vitamins cyanocobalamin, nicotinamide, biotin, ascorbic acid, thiamine, riboflavin and pyridoxine [136].

2.4.4.1 Carbohydrate Content in *R. coriaria*

Glucose, sucrose, fructose and xylose are the core carbohydrates that are found in different parts of *R. coriaria* organs. This fruit is considered as a dietic sugar source called xylitol [137][138]. Fruit pulp and its skin is considered as a main source of carbohydrates. Study reported presence of glucose (0-0.75 %), sucrose (1.41% - 5.85%) fructose (0-1.93%), and xylose (8.53%- 18.19%) in 15 different genotypes of *R. coriaria*. Studies have reported that xylose is one of the major carbohydrates found in all genotypes. A study reported that Xylan (17.07%-27.90%) and glucan (11.27% - 16.73%) are present in great amount while small concentration of galactan (2.43%-4.13) was found. The significant amount of xylose in *R. coriaria* is making it more usable for the production of xylitol, which is used in various industrial products and foods [137].

2.4.4.2 Phytochemical Composition of *R. coriaria*

R. coriaria is found to be enriched in phytochemical constituents. Earliest studies on leaves extract identified the myricetin and gallic acid in 1896 [135]. After that many other useful phytochemicals were also found in other parts of this plant. Previously a extensive study investigated the complete phytochemical profile of the fruit extract and reported 211 phytoconstituents. Since than *R. coriaria* was researched and reported with other components in different parts of plant. Study reported phytoconstituents in fruit extracts with tannins, (iso)flavonoids, terpenoids, anthocyanins and many others (Figure 2.6). Out of 211, the 180 compounds were identified in *R. coriaria* fruits [139]. Several studies also reported and revealed to have more than 250 bioactive constituents from different parts of

R. coriaria [140][141]. In these compounds, phenolic are the biggest class of compounds. The advance analytical techniques including GC-MS and LC-MS/MS are being used in identification of different phytochemicals found in Sumac parts, these include tannins, organic acids, phenolics (flavonoids, phenolic acids), volatiles and some polysaccharides in Table 2.4 [138]. Among all phytochemical constituents, flavonoids were dominant compounds qualitatively in *R. coriaria*. Above 200 phytochemical bioactive components were investigated by utilizing HPLC-DDAD-ESI-MS/MS methods approximately in fruit part [126]. With numerous reports of possible health advantages, the phytochemical constituents in *R. coriaria* have still not been completely known.

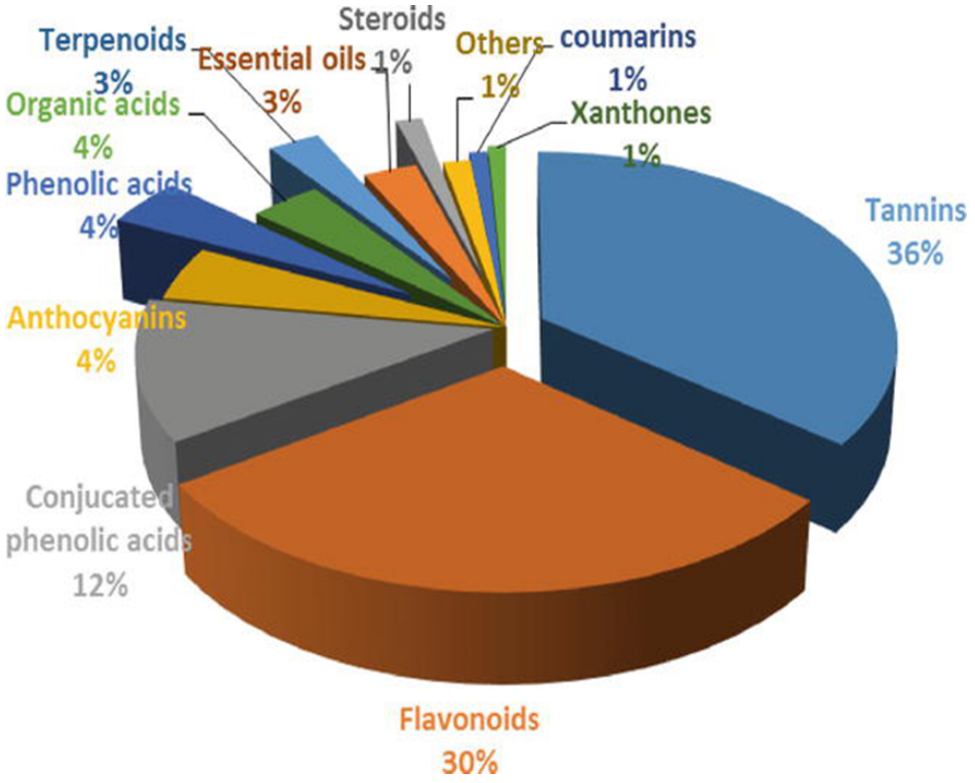


FIGURE 2.6: Chemical composition of *R. coriaria* [128].

TABLE 2.4: Major phytochemical composition in *R. coriaria* plants and essential oils [142].

Sr no	***R. coriaria* parts**	**Compounds**	**Amount (mg / kg)**
1	Fruit and leaves	1. Quercetin	0.231–0.336
	Flavonoids (mg/g)	2. Myricetin	0.316–1.082

Table 2.4 continued from previous page

Sr no	*R. coriaria* parts	Compounds	Amount (mg / kg)
		3. Kaempferol	0.032–0.170
		4. Epicatechin	0.035–0.193
		5. Catechin	0.024–1.300
		6. Quercetin - 3 - O - glucose	0.010–0.136
		7. Rutin	28.42–111.03
		8. Narirutin	10.86–93.99
2	Fruits	1. Delphinidin-3-glucoside	2.8
	Anthocyanins (mg/g)	2. Cyanidin-3-glucoside	0.043–78.400
		3. Cyanidin-3-rutinoside	0.012–0.057
		4. Pelargonidin-3-glucoside	0.381–1.184
		5. Cyanidin chloride	0.015–0.055
		6. 7 - methyl - cyanidin -3 - galactoside	529.2
		7. 7 - methyl - cyanidin - 3 (2"galloyl) - galactoside	3.51
3	Fruits and leaves	1. Gallic acid	0.29–12.04
	Phenolic acids (mg/g)	2. Rosemarinic acid	0.011–0.217
		3. Chlorogenic acid	0.005–1.160
		4. Vallinic acid	0.007–0.022
		5. Caffeic acid	0.001–0.100
		6. p-Coumaric acid	0.002–0.410
		7. Ferulic acid	0.001–0.034
		8. Salicylic acid	1.07–5.97
		9. Syringic acid	0.002–0.010
4	Fruit	1. Malic acid	1360.0–2800.0
	Organic acids (μg/g)	2. Oxalic acid	1.30–2.90
		3. L-Ascorbic acid	4.03–21.40
		4. Citric acid	49.80–95.10
		5. Fumaric acid	0.41–3.40
		6. Tartaric acid	1.20–2.15
5	Fruit and essential oils	1. β-Caryophyllene	40.2–137.9

Table 2.4 continued from previous page

Sr no	*R. coriaria* parts	Compounds	Amount (mg / kg)
	Volatiles (mg/g)	2. α-Pinene	2–260
		3. E-caryophyllene	59–503
		4. Cembrene	19–217
		5. Camphor	3–125
		6. n-Nonanal	18–233
		7. (2E,4E) decadienal	24–165
		8. Nonanoic acid	158

2.4.5 Economic Uses of *R. coriaria*

Many herbal plants are used enormously in industries from earlier period of time. In past times leaves, roots, skin and branches of *R. coriaria* were used as a natural dying. Industries have been using it due to its antioxidant properties to prevent the fat degradation by hindering the development of free radicals. Butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), and propyl gallate (PG) are among the several synthetic antioxidants that are widely utilized. Although consuming these artificial antioxidants may have adverse consequences [143]. Study suggested that *R. coriaria* have beneficial health effects and it is considered as a safe option of antioxidant [144].

R. coriaria belongs to the family of cashew and Pistachio trees. It is grown in tropical regions and Mediterranean basin in middle east and southern Europe. It is also found in South Eastern and Northern regions of Turkey and Lebanon [145]. In the Middle East, it has been valued for its nutritional and medicinal properties since ancient times. It is used in oriental cuisines due to its lemon flavor. These flavor helps in digestion and simulate appetite [141]. Romans and Greeks since ancient times used *R. coriaria* in replacement of lemon and vinegar because of its capability to digest food along with febrifuge properties. Traditional medicine utilizes essential oils for digestive and urinary tract disorders as well as chest pain [139].

R. coriaria stimulates sweating and helps to fight against fever and intestinal irritation. It is also beneficial for skin infections. Because of its antibacterial properties, it can be utilized as an astringent medicinal plant to soothe sore throats [131]. White *R. coriaria*, which is a related specie of red Sumac found in United states in temperate regions where it is utilized by Americans against mouth sores and hemorrhoids. This is a very effective treatment for rheumatism. Additionally, this herb possesses powerful hypoglycemic characteristics that may improve glucose tolerance and lower blood sugar levels [126]. The ability of leaves to dilate blood vessels makes it a protecting agent for cardiovascular system. It possess antioxidant properties that proves to be helpful in DNA protection. In another study Korkmaz also reported the potential of Sumac plant for COVID-19 therapy.

It is a well-known spice which improves the flavor of many meals particularly paired with oregano. Study stated that *R. coriaria* can have a potential economical use. 100g of grinded and dried *R. coriaria* are sold for about 5 USD. While the young *R. coriaria* plant cost about 3 USD, 20 seeds cost approximately about 2 USD. This affirms the real potential of the Sumac for the planters, processors and all other stakeholders. Although *R. coriaria* has useful pharmaceutical, therapeutic, culinary and monetary benefits, however it is very vital to take some precautions while using it due to potential toxicity of leaves [141]. It is recommended to harvest and prepare *R. coriaria* with an expert advice. This standardized preparation of dried *R. coriaria* plant parts may help to achieve the maximum benefits.

2.4.6 Traditional Usages

A wide range of important metabolites that are acting as nutrients and pharmacology including tannins, phenolics, organic acids essential oils and fatty acids have been isolated from *R. coriaria*. Its use for the treatment of stroke is mentioned in book “Cannon of Medicine” written by Avicenna Ibn Sina. *R. coriaria* has also been in use as traditional medicine in treating Ulcers, Diarrhea, Stomach ache, Hemorrhoids pain, Anorexia, Measles, Hemoptysis, Leukorrhea, Dermatitis, Ocular, and Liver Disease. [146]. There are further reports of additional therapeutic applications, such as skin care, digestive tract treatment, headaches, weight

loss, and fever reduction. [147]. In some Iranian traditional medicine, *R. coriaria* showed antidiarrheal, tracheal treatment, hemostasis factor along with prevention of small pox of eye [144].

R. coriaria inhibits the enzyme activity of α-amylase significantly. This enzyme is responsible for breakdown of starch into simpler sugar. So, inhibiting the respective enzyme will restrict the tolerance of glucose in diabetic patients. Additionally, *R. coriaria* can inhibit the xanthine oxidase indirectly which will also reduce blood uric acid in gout patients. It has shown remarkable and exceptional properties as antimicrobial effects after the increase in emergence of microbial resistance [132]. Studies shown that *R. coriaria* can reduce uric acid and blood sugar levels [148].

2.4.7 Medicinal Uses of *R. coriaria*

R. coriaria has been in use as a therapeutic plant and a famous spice since times. This medicinal property of the plant is because of presence of many bioactive components that include flavonoids, hydrolysable tannins, anthocyanins, terpenoids, phenolic acids, organic acids etc. [149]. Alternate scientific names are Rhus glabra L., Anacardiaceae, and Cashew family with common names of Pennsylvania Sumach, Scarlet Sumac, Blue Glabrum, Dwarf Sumac, Sleek Sumach, Smooth Sumac, Upland Sumach, Mountain Sumac.

2.4.7.1 Antibacterial Activities

Now a days, the existing bacterial species are becoming more resistant towards the antibiotics. This resistance is posing the serious threat to mankind in terms of health [150]. We can use alternatives instead of antibiotics to treat bacterial infections by using new antibacterial agents that can act against bacteria more novelly. Using plants as a natural source is becoming an increasingly common approach for preventing people from various kinds of illnesses [151]. The antibacterial activity of many essential oils has been reported by *R. coriaria* against Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus or Bacillus subtilis with

concentrations of 2, 3, or 15 mg/mL respectively [152]. Additionally, *R. coriaria* ethanolic and aqueous extracts were utilized to determine antibacterial activity [158]. The ethanolic extract of *R. coriaria* fruits has showed strong concentration independent antimicrobial activity against broad spectrum of gram negative and gram-positive bacteria. Among all has shown the maximum sensitivity towards fruit extract by giving minimum inhibitory concentration MIC of <0.78%. The similar results of *R. coriaria*'s ripped and unripe fruit ethanolic extract against gram-positive and gram-negative bacteria include Bacillus cereus, Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, Shigella dysentariae, Staphylococcus epidermidis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Yersinia enterocolitica, and Enterococcus faecalis [153]. These results confirms that ancient usage of *R. coriaria* for the treatment as antimicrobial substance can be employed.

2.4.7.2 Antifungal Activities

Fungus is considered as one of the most stubborn pathogens that can infect humans, animals as well as plants. The fungal activity can ruin crops by causing several diseases. Colletotrichum acutatum has ability to attack the temperate plants by damaging immature and mature fruits of plant [154]. Amazingly, *R. coriaria* fruit aqueous extract has demonstrated strong antifungal activity against tomatoes Colletotrichum acutatum causes Anthracnose in tomato plants and fruits [155]. A study reported the antifungal activity of extract from *R. coriaria* against Saccharomyces cerevisiae, Kluyveromyces lactis and Pichia pastoris [156]. The MIC was measured for the fungal strains found to be between 5200 to 7000 μg/ml [157].

2.4.7.3 Antidiabetic

Diabetic mellitus is a metabolic disease which is characterized by hyperglycemic [158] [159]. There are multiple factors which are contributing to the diabetes

including insulin dysfunction, oxidative stress, impaired glucose tolerance, inflammation. These factors eventually result in an altered homeostasis and ends up in T2DM [160]. Many researches revealed that *R. coriaria* has an antidiabetic potential with different extracts. These reports include blood biomarkers, insulin resistance, glucose metabolizing enzymes [131] [161]. A report from in vitro investigation discovered that ethyl acetate extract of *R. coriaria* fruit has shown the antidiabetic potential by inhibition the activity of alpha- amylase [162]. A study has shown results related to the treatment of diabetic rats by giving lyophilized hydrophilic extract of fruit of *R. coriaria* plant for 3 weeks orally. This resulted in significant decrease in serum of glucose, LDL, TC, TG, HDL [163]. Interestingly some studies reported, that *R. coriaria* has produced a significant decrease in level of glycated hemoglobin (HbA1c) and alpha – glucosidase activity and as a result insulin levels were seen reduced in animals' serum. In another study diabetic rats were treated with *R. coriaria* extract and resulted in reduced alkaline phosphate activity with LDL levels and serum glucose [164]. The authors of a different studies have reported the similar results against diabetes. There was drop in serum glucose and insulin resistance after giving *R. coriaria* powder as a dietary supplement in case of diabetes 2 diabetic women. This supplementation also worked as antioxidant in diet [165]. Additionally, a group of patients with diabetes type 2 after consuming Sumac powder in quantity of 3.0 grams have shown a decrease in glucose blood serum along with significant increase in HbA1-c and Apo-B [166].

2.4.7.4 Cardioprotective

R. coriaria has also shown cardiovascular activity. Research reported that by utilizing methanolic extract of leaves to rabbits have shown to protect the heart by inhibitory action of tumor necrosis factor -α (TNF-α) and eliminating free radicals and reactive species with the stimulation of cyclooxygenase (COX) pathway. Polygalloyloted D-glucopyranose with various 3-O-methyl gallic acid and galloloyation can exhibit the cardiovascular activities against above-mentioned problems [167]. According to reports, hyperlipidemia and the risk of atherosclerosis are

linked. Rats administered a cholesterol-rich diet were given aqueous ethanolic extract from *R. coriaria* fruit, similar to another study. Findings has shown that the extract was efficient to reduce the high serum concentration and resulted in easing hypercholesterolemic conditions in rats [168].

2.4.7.5 Antinociceptive Activities

Pain and unpleasant sensation in body is connected with many kinds of ailments. While presence of pain is an uncomfortable and serious challenge in field of medicine. It's been years that field of medicine is finding out the best possible pain killer which may have fewer or no side effects. Interestingly pretreatment with *R. coriaria* hydroalcoholic extract of leaf has shown an analgesic effect in writhing in Wistar rats [169]. This lessening in writhing in rat was connected with the increase in the tail-flick time and blocking of formalin test, suggested an antinociceptive effect from *R. coriaria*. These findings confirm the pain-relieving properties of Sumac through central and peripheral mechanisms [169]. On the basis of above findings, *R. coriaria* has proven to be novel and natural source with analgesic property.

2.4.7.6 Neuroprotective Activities

Neurodegenerative disorders are most commonly caused by various traumatic injuries which include neuroinflammation and hypoxia generating oxidative stress. These injuries are associated with Alzheimer's disease such as optic neuropathies [170]. Visual impairment and blindness can be caused by retinal degeneration. This degeneration happens when the retina's blood supply is inadequate to support the metabolic needs of the eye [171] [172].

Consequently, neuroprotective medicine with some beneficial approaches is needed to clock, reverse or decrease the neuronal cell death in neurodegenerative illnesses. The ethanolic extract of *R. coriaria* fruit was found to have neuroprotective effects against retinal degeneration in vitro for rat retinal ganglion cell lines RGC-5 [170].

Outcomes of study have shown that ERC significantly decreased the serum deficiency made death of RGC-5 cells. Results shown that ERC greatly decreased the death of RGC-5 cells caused by serum deprivation. Additionally, it was discovered that ERC completely eliminated the decrease in GST and GSH levels brought on by serum deprivation[170].

Results support the use of *R. coriaria* in traditional medicine by highlighting a possible neuroprotective action against retinal degeneration. The neuroprotective properties of sumac fruit should be further confirmed by testing more brain cell lines and doing animal research.

2.4.7.7 Anticancer Activities

Several researches on *R. coriaria* have provided the evidence for the inhibitory role on growth of tumors and survival [173][153]. El Hasasna et al has reported that *R. coriaria* extracts have seen to promote autophagic cell death, senescence and suppressed cell migration. These reported effects were also verified later by in vivo testing the growth assay for embryonic tumors [174].

Researchers discussed the mechanism for anticancer activity which includes inhibition of STAT3, NFB and NO pathways by using *R. coriaria* extract. Two in vivo models including (chemically induced rat mammary carcinogenesis model) and (mice 4T1 adenocarcinoma allograft model) were administered with *R. coriaria*.

This has induced strong therapeutic and chemo preventive potential through antiproliferative, epigenetic alteration, antiangiogenic and pro-apoptic alterations [167]. *R. coriaria* extract treatment reduced the development of human colorectal cancer cells Caco-2 and HT-29 [175].

In another study, *R. coriaria* extract has also induced Beclin-1-inddependet autophagy, activation of autophagy, caspase-7- dependent apoptosis and deactivation of the AKT/mTOR pathway by degradation of both proteins which is proteasome dependent.

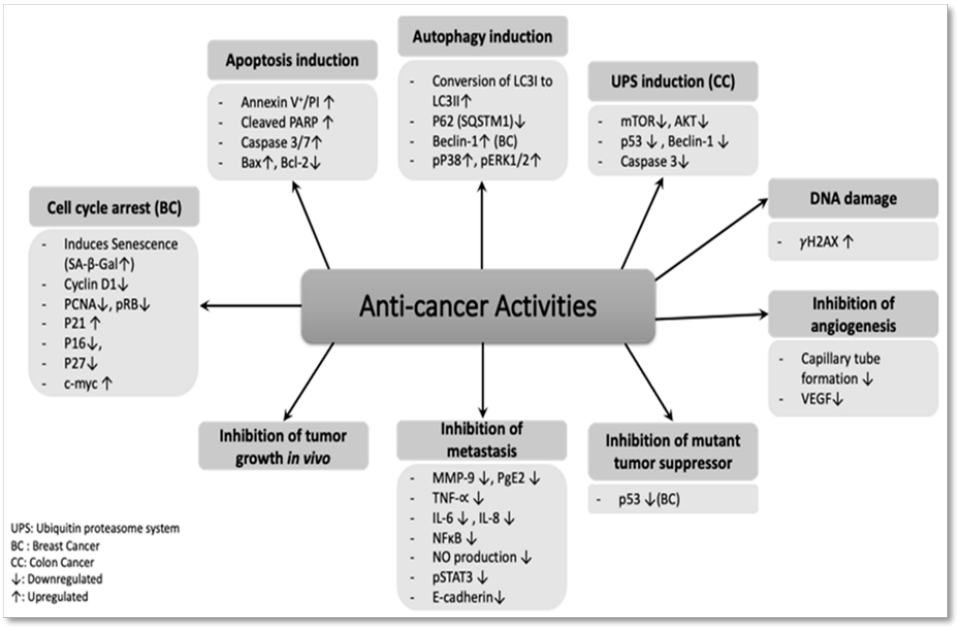


FIGURE 2.7: A summary of molecular targets of *R. coriaria* against cancer [141].

2.4.7.8 Dental Protection Activities

The integrity of resin dentin connections created with modern adhesives is lost due to enzymatic breakdown of the dentin matrices [176]. Matrix metalloproteinases MMMPs are endogenous proteases in charge of the collagen tissues turnover [177].

Despite MMPs' inactivity in mineralized dentin, the use of etching acids or acidic monomers can reveal and initiate these proteases, leading to a gradual loss of collagen from the tooth's composite layers [178]. Researchers investigated the MMP activity of demineralized dentin matrix following pretreatment using multiple collagen crosslinkers, including fruit extract from *R. coriaria*. When compared to the control, this extract dramatically decreased the total dentin MMP activity [169].

Furthermore, a reduction in MMP-2 and MMP-9 activity was demonstrated by zymogram examination of dentin powder mixed with *R. coriaria* extract. Moreover, MMP-8, MMP-2, and MMP-9 release was decreased in extracts of dentin treated with *R. coriaria*, according to multiplex bead analysis [169]. As a result, *R. coriaria* could provide fresh MMP inhibitors that might be applied to dental disease prevention or therapy.

2.4.7.9 Antifertility

Reproductive system of human can easily be impacted by many pathological factors. These modifications can end up in different subfertility or entire infertility problems. Throughout the ongoing development of reproduction, any alteration in the molecular structure can lead to abnormal functioning of female and male reproductive system [179]. Several studies have informed that one in seven couples around the world is found infertile [180].

Roshan khan *et al.* reported that *R. coriaria* has shown the properties that can enhance the male infertility. *R. coriaria* extract also increased testosterone level, TAC level, inflammatory cytokine with the inhibition of p53 expression and caspase gene 3. The administration of extract can be used as therapeutic effect in terms of scavenging the effect of morphine impacts in sixty-two rats [142].

2.4.7.10 Antiviral Activity

About twenty-five plant species having medicinal properties found in Iran were studied among which aqueous extract of *R. coriaria* has shown important antiviral activities against HSV-1 and adenovirus type 5 [181]. Interestingly four biflavones including amentoflavone, sumaflavone, hinokiflavone and agathisflavone were extracted from fruits and leaves of many different species of Rhus species.

While Amentoflavone and agathisflavone shown the antiviral activity against influenza viruses (type A and B). Amentoflavone have shown slight anti HSV-1 and HSV-2 activities with EC50=18 and 48 μg/mL, respectively [182].

In another study, hinokiflavone, amentoflavone, and agathisflavone have shown good antiviral activities against HIV-1 reverse transcriptase from 65-100 μM with value range of IC50 [183]. On the other hand, researchers also found that hinokiflavone which was isolated from Podocarpus macrophyela also shown a same kind of activity. This activity was shown in Raji cells' production of the Epstein-Barr virus genome, indicating the antiviral potential of biflavones [184].

2.5 Toxicology Studies on *R. coriaria*

From centuries, herbal remedy has been in use by human beings because of their less toxicity and enhanced safety for treatment of many diseases. However, some recent studies have reported about the adverse effect of using herbal medicines as well [185]. Therefore, it is necessary to assess the toxicological profile of any of the useful plants extract for medicinal use for human beings. While *R. coriaria* has been considered as safe to use by animals and human. Researchers studied the therapeutic effect of *R. coriaria* on diabetic which was fortified by streptozotocin. Research was conducted to check the toxicity by using 3 different doses (250, 500 and 1000mg/kg) of Sumac extract. The results show that *R. coriaria* is non-lethal by oral uptake even at high dose. It was confirmed by administration of the extract for three days [163]. Moreover, a study was done by administrating *R. coriaria* seed extract to a diabetic mice orally in amount of 300mg/kg and as a result type 2 diabetic mice show good results without any harmful effects [186]. Janbaz *et al.,* stated that the 5g/kg crude extract of *R. coriaria* has shown antidiarrheal and antisecretory effect in mice [187]. A group shared that a chick embryo was given 150g/ml of ethanolic extract of this plant can inhibit the breast cancer growth [174]. So, from existing literature it has been proved that this plant is very safe to use and is considered more attractive for medicinal drug in treatments.

2.6 Molecular Docking

It is now possible to discover novel potential therapeutics against many diseases with the help of molecular docking from small molecule databases. Molecular docking is basically a computational modeling that gives interaction between receptor and ligand complex [188]. Molecular docking is a technique that gives best possible binding affinities, best pose of ligands and the association of protein and ligand by forming a complex. Orientation of ligand can be helpful to give the information about the target binding site of receptor that can bind to a ligand through best scoring function. Input in the docking process are the 3D structures

of ligands and proteins while we get the output in form of binding affinities to predict the best docked structures for therapeutical purposes [189]. Moreover, we can also find out the essential properties along with highest binding affinities, the reasonable mechanism of absorption, excretion, distribution, and metabolism to select the best lead compound in researches [190]. One of the main key areas of molecular docking is determining the protein and ligand complex for drug discovery process. Molecular docking can be performed by many software available online and offline. These include PyRx, Auto dock, CB dock, Auto dock vina, Patch dock etc. While the most common factors that are measured in software are binding affinities, types of bonds, bond size and many more [191].

2.7 Gap Analysis

Current research confirms Rhus coriaria (Sumac) has broad antiviral and anti-inflammatory properties against viruses like influenza, but there is a complete absence of direct studies testing it against Human Metapneumovirus (HMPV) . The critical gap is the lack of specific insilico docking and simulation studies to predict binding affinity with HMPV viral proteins, alongside missing in-vivo (animal) evidence for efficacy against this specific pathogen—meaning its effectiveness remains hypothetical and unproven for HMPV.

Chapter 3

Methodology

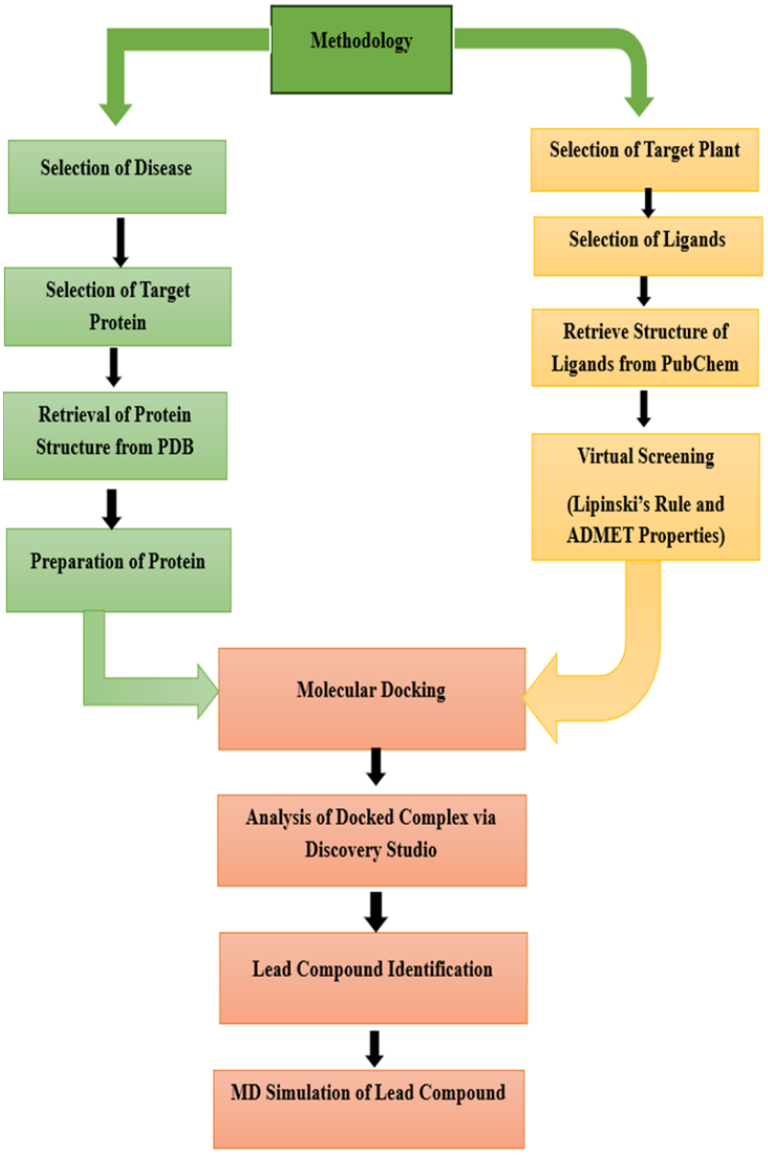


FIGURE 3.1: Flow Chart for Methodology

3.1 Databases and Tools

3.1.1 Databases

3.1.1.1 PubMed

PubMed is a cost-free database which is maintained by National Library of Medicine (NLM) and National Centre for Biotechnology Information (NCBI) of United States of America (pubmed.ncbi.nlm.nih.gov) [192].

It helps user to find out the articles of their interest. It provides access to a wide variety of publications related to various disciplines. It offers thorough search features, direct access to the complete text, and citation information for every article.

3.1.1.2 Google Scholar

It is an online search engine made to find academic books, theses, conference papers, articles, and patents. It suggests full-text article links, citation tracking, author biographies, notifications, complex search filters, Google Scholar metrics, and comprehensive coverage across several fields (scholar.google.com) [193].

3.1.1.3 PDB

PDB is a worldwide well-known resource for retrieval of 3D structural data of biomolecules (www.rcsb.org) [194]. This manages and stores data of proteins and nucleic acids compromising NMR, crystallographic and EM structures. PDB provides free access to data by user friendly website with various download options. The data is stored in standardized file known as PDB format.

The PDB is regularly updated and integrated with software tools, making it a vital resource for the global scientific community.

3.1.1.4 PubChem

Pub chem is a publicly available database which gives the comprehensive information about the chemicals including their properties, synthesis, patents, drug labeling, spectrum information, clinical studies, and structure of molecules (pubchem.ncbi.nlm.nih.gov/) [195].

3.1.1.5 PkCSM

(pkCSM) is a tool that uses graph-based signatures to develop predictive models of vital ADMET properties for drug development. It can be accessed cost free on web server which holds no information submitted to it and delivers a platform to quickly assess the toxicity and pharmacokinetic properties. (structure.bioc.cam.ac.uk/pkcsm) [196].

3.1.2 Tools

3.1.2.1 BIOVIA Discovery Studio Visualizer

Discovery Studio is a user-friendly software which can provide the 2D and 3D images of protein and ligand complex. This gives a detail image of all kinds of interactions that are formed inside the complex. Proteins along with other small data can be observed, shared, and analyzed using this feature-rich, free molecular modeling software.

As visualization is a key tool for evaluation and dissemination of modeling research, this tool was used for visualization and analysis purpose in current research [197] (www.3ds.com/biovia/visualization).

3.1.2.2 PyRx

PyRx is a virtual screening tool used for computational drug discovery to investigate large sets of compounds against drug targets. It assists user at every step

from preparing data to analysis completely. It is an easy-to-use interface making a valuable tool in researches for drug discovery.

PyRx offers docking tools, spreadsheets style features and strong visualizing capabilities making it suitable for structure-based drug design.

This software uses multiple ligands to perform molecular docking and gives the best pose of each ligand individually that binds to the protein. The results are evaluated on the basis of inspection of ligand position and quantitatively by observing the scoring algorithms.

Maximum binding energy is observed in case of successful docking [198] (https://pyrx.sourceforge.io/).

3.1.2.3 Schrödinger

Schrödinger software is a computational software widely used for performing molecular modeling and simulations. This software uses quantum mechanics, molecular dynamics and docking tools to support drug discovery and material researches.

The platform provides the user accurate prediction about molecular structures, properties and interactions aiding in scientific research (Schrödinger, Inc., 2023) [199].

3.2 Selection of Disease

Human metapneumovirus hMPV is a main reason of respiratory tract infections. This disease is common from infants to elders and people with compromised immunity globally [54].

Since, the hMPV was discovered in 2001, there is no vaccine found or any other antiviral treatment to treat this illness. This virus causes the symptoms of severe respiratory sicknesses including pneumonia and bronchiolitis [32].

3.2.1 Selection of Target Protein

Protein that is involved in entry of hMPV into the human body is primarily known as F protein. It is also called as fusion glycoprotein denoted as F0 [200].

The highly conserved RGD motif found in hMPV F is typical of proteins that attaches to integrins, recommending that integrins might be hMPV F's functional receptors.

Integrins enhance infectivity of hMPV through an unidentified mechanism, showing that after first binding to proteoglycans, hMPV F interacts with integrins. [87].

3.2.1.1 Reasons for Selecting the F Protein of hMPV as a Drug Target

i. Essential Role in Viral Entry and Infectivity

F protein's main function is to facilitate the fusion of the viral envelope into the host membrane of the cell. The viral life cycle has just initiated. The metastable prefusion state of the F protein is present. It undergoes a significant conformational shift to a stable post-fusion state upon triggering, which propels the membrane merging.

The virus is efficiently neutralized by preventing this conformational change. Strong fusion inhibitors are medications that either stabilize the prefusion state or prevent the transition to the post fusion state [90].

ii. Highly Conservation Across Viral Strains

The fusion protein is significantly more conserved as compared to other major surface proteins glycoprotein (G) across all linages (A and B) and sub linages. The studies have revealed that drugs which target conserved regions of Fusion protein are expected to be more effective by overcoming the challenge of genetic diversity of virus [42].

iii. Proven Susceptibility to Inhibition Precedent

Inhibitors of Fusion protein has been reported for paramyxoviruses. The well-known example is the use of peptide drug T-20 (Enfuvirtide) for HIV. Several studies on hMPV also have reported that monoclonal antibodies and small molecules have ability to neutralize the virus by binding with F protein. "The hMPV F protein is known to be a feasible drug target, which makes it practical to look for new inhibitors in natural products." [201].

iv. Presence of Functional Sites for Drug Binding

F protein present in hMPV has specific structural sites that are considered important for proper functioning by providing ideal pockets for binding of small molecules and interference. Hydrophobic regions which help in insertion into the host membrane form a stable six helix bundle during fusion that are driving force for merging membrane.

The neutralizing antibodies bind to particular regions of the F protein, highlight vulnerabilities that could be targeted by small compounds [202].

3.3 Retrieval of Target Protein

The PubMed IDs of research articles containing information related to F protein were used as literature review. The 3D structure of protein was confirmed and downloaded in pdb format with (pdb id: pdb_00005WB0) and DOI: doi.org/10.221/pdb5WB0/pdb from PDB.

3.4 Visualization of Protein and Ligands

Discovery Studio Visualizer was used to observe the protein structure and ligands. All structures were saved as PDB files by the help of same software.

3.5 Cleaning of Protein

Discovery Studio Visualizer was used to clean protein by removing water molecules and ligands. These molecules are removed before molecular docking because most crystallographic water and non-essential cofactors can interfere with the ligand and protein binding. Removing extra molecules will simplify and improves the reliability of predicted protein ligand interaction [203].

3.6 Selection of Target Plant

R. coriaria has remain in use as a medicinal plant and a famous spice for times. This medicinal property of the plant is because of many bioactive constituents that contain terpenoids, flavonoids, hydrolysable tannins, anthocyanins, phenolic acids and organic acids etc. [149].

R. coriaria was chosen for possessing several medicinal properties including antibacterial, antifungal, antidiabetic and antiviral activity (specifically against HSV-1 and adenovirus type 5 and type A and B influenza viruses) [204].

3.7 Selection of Ligands

More than 211 phytochemical compounds have been investigated in recent studies from *R. coriaria* but only 30 compounds with widely reported medicinal activities were selected for this study [139]. Table 3.1 shows selected compounds such as phenolics, organic acids, and volatile of *R. coriaria* plant. Among all the bioactive compounds, flavonoids were found to be the most important chemicals in this plant.

The mentioned compounds hold medicinal value. Chemical IDs and Structure of selected compounds were retrieved from the PubChem (https://pubchem.ncbi.nlm.nih.gov/). Structures of selected ligands were downloaded in sdf format.

TABLE 3.1: Chemical compounds names and their phytochemical family [142].

Sr. no	Chemical Compound	Phytochemical Family
1.	Quercetin	Flavonoids
2.	Myricetin	Flavonoids
3.	Kaempferol	Flavonoids
4.	Epicatechin	Flavonoids
5.	Catechin	Flavonoids
6.	Quercetin-3-O-glucose	Flavonoids
7.	Rutin	Flavonoids
8.	Narirutin	Flavonoids
9.	Delphinidin-3-glucoside	Anthocyanins
10.	Cyanidin-3-glucoside	Anthocyanins
11.	Cyanidin-3-rutinoside	Anthocyanins
12.	Pelargonidin-3-glucoside	Anthocyanins
13.	Cyanidin chloride	Anthocyanins
14.	Gallic acid	Phenolic Acid
15.	Rosmarinic acid	Phenolic Acid
16.	Chlorogenic acid	Phenolic Acid
17.	Caffeic acid	Phenolic Acid
18.	p-Coumaric acid	Phenolic Acid
19.	Ferulic acid	Phenolic Acid
20.	Salicylic acid	Phenolic Acid
21.	Syringic acid	Phenolic Acid
22.	Malic acid	Organic Acid
23.	L-Ascorbic acid	Organic Acid
24.	Citric acid	Organic Acid
25.	Tartaric acid	Organic Acid
26.	E-caryophyllene	Volatiles
27.	Cembrene	Volatiles
28.	Camphor	Volatiles
29.	Sumaflavone	Volatiles

Table 3.1 continued from previous page

Sr no	Chemical Compound	Phytochemical Family
30.	Amentoflavone	Volatiles

3.8 Virtual Screening

3.8.1 Lipinski Rule

Lipinski's rule of 5 (RO5) is a physiochemical parameter that should be met by any ideal therapeutic molecule. It gives the confirmation about the characteristics of any chemical molecule that is suitable for oral administration to carry certain biological functions in our body. If any selected compound or molecule satisfies the characteristics of Lipinski's rule it will show enhanced pharmacokinetics' characteristics in an organism during metabolic process [205]. As per Lipinski's rule of five, an oral medication shouldn't go against more than one of the of the following:

TABLE 3.2: The following are the Lipinski's rule of five.

Sr no.	Parameter	Standard
01	Hydrogen bond acceptors	≤ 10
02	Hydrogen bond donors	≤ 5
03	Molecular weight	≤ 500 g/mol.
04	LogP	<5
05	Rotatable bonds	<10

PkCSM omictools.com/pkcsm-tool, an online tool was used to evaluate the compounds for Lipinski Rule of Five.

3.8.2 ADMET Properties

In addition to biological activity, drugs therapeutic effect is also dependent on a suitable ADMET profile [206]. Pre clinic optimization includes the physiochemical properties and in silico toxicity assessment that are essential to meet the criteria

for use of any molecule as a nutraceutical drug against any disease. A compound's ADMET properties are determined by its pharmacokinetic profile. PkCSM is used to find out the pharmacokinetics and toxicity parameters of chemical compounds [207].

By using pkCSM online server, prediction about the phytochemicals were made. To search for the ADMET properties SMILES of chosen compounds were uploaded, and as a result, absorption, distribution, metabolism, excretion and toxicity were predicted [208].

3.9 Molecular Docking

The target protein, after cleaning, was uploaded in PyRx software for docking. The file format pdb was converted to pdbqt. Selected ligands were also uploaded in PyRx. Babel in PyRx was used to minimize the energy of ligands and for the conversion of ligand file format pdb to pdbqt.

By using the Vina wizard, grid size was maximized and adjusted according to ligand and protein. Vina was used to perform molecular docking.

3.10 Visualization and Analysis of Protein Ligand Interaction

Interactions between the ligand and the targeted protein were observed for the purpose of analysis of the docking results. Compounds having the strongest interaction with the specific protein were chosen after docking.

These ligand-protein interactions were examined using the desktop visualization program Discovery Studio Visualizer. Discovery Studio received the docked protein in PDB format. Discovery Studio was used to observe complex proteins through determining the relationship of the residue with the ligand.

3.11 Lead Compound Identification

Virtual screening for Lead compound was done by Lipinski's rule of 5 and ADMET properties analysis. Further PyRx was utilized for molecular docking of screened compounds with target Fusion protein to observe their binding affinities. Chemical interactions (Hydrogen, Electrostatic and Hydrophobic) between amino acids of protein and ligands were also studied. All molecular transactions within and between cells require noncovalent interactions between molecules of various sizes and kinds, which are common in cellular processes. These interactions affect binding affinity, which is crucial for therapeutic efficacy, as well as characteristics like drug potency [209]. The compounds that satisfy all of the above-mentioned criteria were chosen as Lead compound.

3.12 Molecular Dynamic Simulation

The MD Simulation of shortlisted lead compounds was performed to assess the conformational stability of the protein ligand complex by employing Schrödinger software. The MD simulations were executed at (NPT ensemble, 310 K, 1 atm) for 200 nanoseconds. Na^+ and Cl^- ions at a salt concentration close to physiological ($\sim$51 mM) were added to neutralize the system after the system was dissolved in a water box contained approximately 20,093 water molecules. The Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) plots were retrieved in PNG format for further analysis using the simulation interaction diagram module [199].

Chapter 4

Results

4.1 Selection and Preparation of Protein

4.1.1 Structure of Protein

Three-dimensional structure of Fusion protein was retrieved from protein data bank. Sequence length of viral Fusion protein was 542 with resolution of 2.60 Å. PDB provided the structure of fusion protein shown in (Fig 4.1) with the pdb id: pdb_00005wb0.

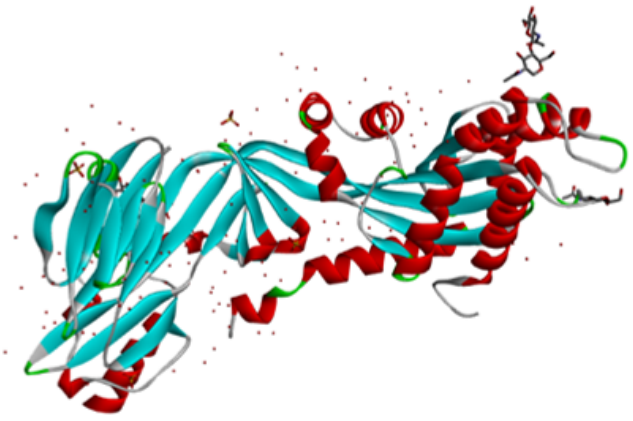


FIGURE 4.1: Structure of Fusion protein (F0) retrieved from PDB

4.1.2 Cleaning of Protein

The Fusion protein acquired from PDB was refined to prepare it for molecular docking (Fig 4.2). For this purpose, Biovia Discovery Studio Visualizer was used to remove water molecules and heteroatoms. These molecules are removed before molecular docking because most crystallographic water and non-essential cofactors can interfere with the ligand and protein binding. Removing extra molecules will simplify and improves the reliability of predicted protein ligand interaction [203]. After cleaning of protein, it was saved in PDB format.

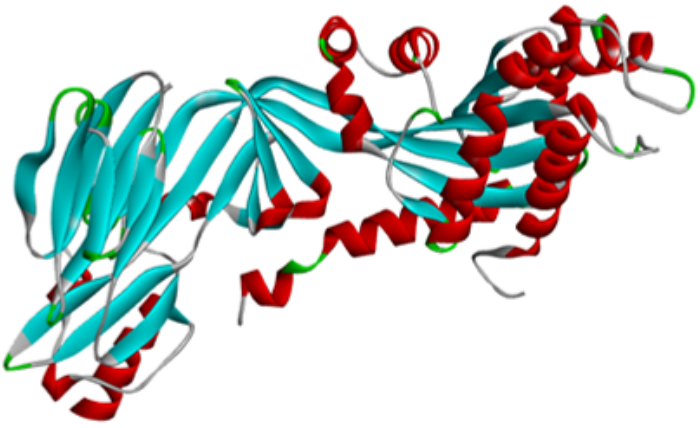


FIGURE 4.2: Structure of Fusion protein (F0) after cleaning from Discovery Studio Tool

4.1.3 Ligands Selection and Preparation

PubChem is an open database that provides information about chemical structures, properties and biological activity. This database is handled by National Institutes of Health (NIH). It focuses on small molecules but also gives data for many macromolecules like peptides, lipids, carbohydrate, nucleotides etc [210].

A library list of 30 bioactive compounds was prepared from literature review, and their respective structures were retrieved from PubChem in SDF format. Table 4.1 shows molecular weight, formula and structure of all ligands obtained from

PubChem with IDs. By using Discovery Studio ligands were converted in PDB format. This software was also used for visualization of 3D structures of ligands.

TABLE 4.1: Ligands selected from *R. coriaria* with their PubChem ID's and 3D structures retrieved from PubChem.

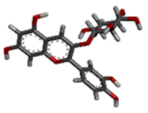
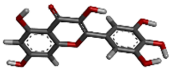
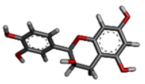
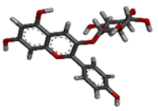
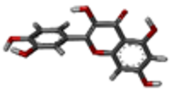
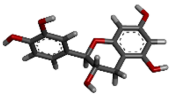
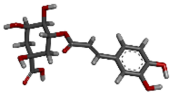
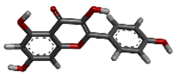
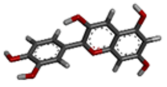
Sr. No.	**Ligands**	**Molecular Formula**	**Molecular Weight**	**PubChem ID**	**3D Structure**
1.	Cyanidin-3-glucoside	$C_{21}H_{21}ClO_{11}$	484.841	197081	
2.	Myricetin	$C_{15}H_{10}O_8$	318.237	5281672	
3.	Epicatechin	$C_{15}H_{14}O_6$	290.271	72276	
4.	Pelargonidin-3-glucoside	$C_{21}H_{21}ClO_{10}$	468.842	3080714	
5.	Quercetin	$C_{15}H_{10}O_7$	302.238	5280343	
6.	Catechin	$C_{15}H_{14}O_6$	290.271	9064	
7.	Chlorogenic acid	$C_{16}H_{18}O_9$	354.311	1794427	
8.	Kaempferol	$C_{15}H_{10}O_6$	286.239	5280863	
9.	Cyanidin Chloride	$C_{15}H_{11}ClO_6$	322.7	68247	

Table 4.1 continued from previous page

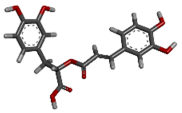
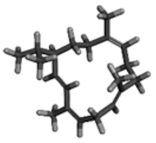
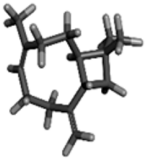
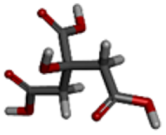
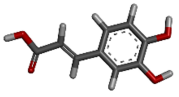
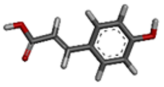
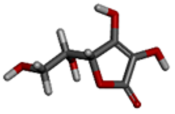
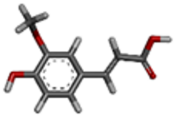
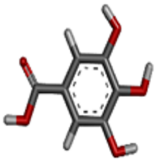
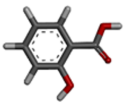
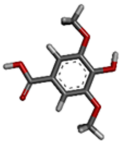
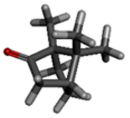
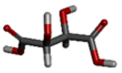
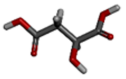
Sr. No.	Ligands	Molecular Formula	Molecular Weight	PubChem ID	3D Structure
10.	Rosmarinic acid	$C_{18}H_{16}O_8$	360.318	5315615	
11.	Cembrene	$C_{20}H_{32}$	272.476	6430770	
12.	E-caryophyllene	$C_{15}H_{24}$	204.357	5281522	
13.	Citric acid	$C_6H_8O_7$	192.123	311	
14.	Caffeic acid	$C_9H_8O_4$	180.159	689043	
15.	p-coumaric acid	$C_9H_8O_3$	164.16	637542	
16.	L-Ascorbic acid	$C_6H_8O_6$	176.124	54670067	
17.	Ferulic acid	$C_{10}H_{10}O_4$	194.186	445858	
18.	Gallic acid	$C_7H_6O_5$	170.12	370	

Table 4.1 continued from previous page

Sr. No.	Ligands	Molecular Formula	Molecular Weight	PubChem ID	3D Structure
19.	Salicylic acid	$C_7H_6O_3$	138.122	338	
20.	Syringic acid	$C_9H_{10}O_5$	198.174	10742	
21.	Camphor	$C_{10}H_{16}O$	152.237	2537	
22.	Tartaric acid	$C_4H_6O_6$	150.086	444305	
23.	Malic acid	$C_4H_6O_5$	134.087	525	

4.2 Screening of Bioactive Compounds for Selection of Lead compound

4.2.1 Virtual Screening through Lipinski Rule of Five

To Prioritize the lead compound, initial evaluation by Lipinski's rule of 5 was performed. After Lipinski's rule of 5 compounds were further evaluated by ADMET properties and binding affinities were used as filter.

For the initial selection of compounds to be used as drug against the disease, Lipinski rule of 5 was applied as the primary step to screen the compounds safe for oral availability [211]. The Lipinski rule deals with certain parameters like Molecular weight which should be ≤ 500, log P ≤ 5, H-bond donors ≤ 5, H-bond acceptors ≤ 10 and Rotatable bond ≤ 10. A compound is considered safe as drug

when it follows 3 or more rules. If it violates two or more rules it is considered as poorly absorbed [212].

This is the foremost and basic filter which screens out the best candidates for oral drug development as bioavailable standpoint. According to table 4.2, among all of the selected 30 ligands (Sumaflavone, Amentoflavone, Narirutin, Rutin, Cyanidin-3-rutinoside, Quercetin-3-O-glucose and Delphinidin-3-glucoside) were not obeying the Lipinski rule. While remaining 23 compounds were found acceptable among them only 4 compounds (Cyanidin-3-glucoside, Myricetin, Pelargonidin-3-glucoside, Chlorogenic acid and Cembrene) were violating one rule but making it appropriate for selection for further analysis. Whereas, rest of all 18 compounds were following all rules as mentioned in table 4.2.

TABLE 4.2: Details of Lipinski Rule of five for the 30 selected ligands.

Sr no.	**Compounds name**	**Mol. Wt. g/mol ≤500**	**Log P ≤5**	**Rotatable bond ≤10**	**HBA ≤10**	**HBD ≤5**	**Yes or No**
1.	Sumaflavone	554.463	4.8396	3	11	7	NO
2.	Amentoflavone	538.464	5.134	3	10	6	NO
3.	Narirutin	580.539	-1.165	6	14	8	NO
4.	Rutin	610.521	-1.6871	6	16	10	NO
5.	Cyanidin-3-rutinoside	595.53	-0.7662	6	14	10	NO
6.	Quercetin-3-O-glucose	506.416	0.0319	5	13	7	NO
7.	Delphinidin-3-glucoside	500.84	-2.9084	4	11	9	NO
8.	Cyanidin-3-glucoside	484.841	-2.614	4	10	8	YES
9	Myricetin	318.237	1.6936	1	8	6	YES
10	Epicatechin	290.271	1.5461	1	6	5	YES
11	Pelargonidin-3-glucoside	468.842	-2.3196	4	9	7	YES
12	Quercetin	302.238	1.988	1	7	5	YES
13	Catechin	290.271	1.5461	1	6	5	YES

Continued to next page

Table 4.2 continued from previous page

Sr no.	Compounds name	Mol. Wt. g/mol ≤500	Log P ≤5	Rotatable bond ≤10	HBA ≤10	HBD ≤5	Yes or No
14	Chlorogenic acid	354.311	-0.6459	4	8	6	YES
15	Kaempferol	286.239	2.2824	1	6	4	YES
16	Cyanidin Chloride	322.7	-0.0871	1	5	5	YES
17	Rosmarinic acid	360.318	1.7613	6	7	5	YES
18	Cembrene	272.476	6.6178	1	0	0	YES
19	E-caryophyllene	204.357	4.7252	0	0	0	YES
20	Citric acid	192.123	-1.2485	5	4	4	YES
21	Caffeic acid	180.159	1.1956	2	3	3	YES
22	p-coumaric acid	164.16	1.49	2	2	2	YES
23	L-Ascorbic acid	176.124	-1.4074	2	6	4	YES
24	Ferulic acid	194.186	1.4986	3	3	2	YES
25	Gallic acid	170.12	0.5016	1	4	4	YES
26	Salicylic acid	138.122	1.0904	1	2	2	YES
27	Syringic acid	198.174	1.1076	3	4	2	YES
28	Camphor	152.237	2.4017	0	1	0	YES
29	Tartaric acid	150.086	-2.1226	3	4	4	YES
30	Malic acid	134.087	-1.0934	3	3	3	YES

4.2.2 ADMET Properties

4.2.2.1 Pharmacokinetics

Pharmacokinetics is a branch of pharmacology which studies the effect of drug on the body and how body reacts when drug enters into it. This deals with analysis of absorption, distribution, metabolism, and excretion of drugs [213].

4.2.2.2 Absorption

The rate and extent to which drug moves from administration point to its target site where it will act, is known as drug absorption. It is a very important phase in

studying drug pharmacokinetics. Drug will take certain time to produce noticeable effects on the body [214]. If the log Papp value of drug or chemical is >0.90cm/s, then it is believed that it may have high level of $CaCO_2$ permeability [214]. Water solubility is also one of the important factor that is required for the reaction of drug. Higher water solubility validates the good features of drug with high absorption and bioavailability by ensuring therapeutic effectiveness of drug [215].

The solubility of compound ranges from -4 to -2, Soluble (-2 to 0), and extremely soluble (> 0). Recommended skin permeability >-2.5cm/h is an important factor for enhanced drug effectiveness. This parameter is important in development of drug with transdermal route of administration [216]. P-glycoprotein is an ABC transporter (an ATP- binding cassette). This acts as biological barrier by elimination of xenobiotics and toxins. P-gp I/II inhibitor is the capability of chemical to stop transportation of P-gp I and P-gp II. There is important pharmacokinetics outcome for P-gp substrates when P-gp mediated transport is modified [216].

According to table 4.3, based on the intestinal absorption values all selected 23 compounds were categorized into 3 main groups high intestinal absorbers (>70%) includes E-caryophyllene (94.8%), Camphor (96.0%), p-coumaric acid (93.5%), Ferulic acid (93.7%), Cembrene (94.4%) are showing best intestinal absorbance because of their smaller size and favorable LogP values. Whereas medium intestinal absorbers (30-70%) include Quercetin (77.2%), Kaempferol (74.3%), Epicatechin/Catechin (68.8%), Myricetin (65.93%) were well absorbed despite of being a P-gp substrates.

Poor absorbers include <30% (Cyanidin-3-glucoside (29.9%), Pelargonidin - 3 - glucoside (32.9%), Chlorogenic acid (36.4%) and Rosmarinic acid (32.5%) have shown poor absorption which is due to its higher molecular weight and polarity. On the other hand, Tartaric acid (3.7%) and Citric acid (0%) are also very poorly absorbed. Out of 23 compounds only 3 compounds Camphor, Cembrene and E-caryophyllene have shown high skin permeability i.e. (-2.002, -1.675. -1.58) respectively. While all other were having low skin permeability as mentioned in table 4.3. Water solubility for all compounds rang from (-1.38 o -3.11) that satisfy criteria of water solubility i.e. (0 to -4) except Cembrene and E-caryophyllene.

TABLE 4.3: Absorption parameters for 23 selected ligands from *R. coriaria*.

Sr no	**Ligand**	**Water solubility**	**$CaCO_2$ permeability**	**Intestinal absorption (human)**	**Skin Permeability**	**P-glycoprotein substrate**	**P-glycoprotein I inhibitor**	**P- glycoprotein II inhibitor**
1.	Epicatechin	-3.117	-0.283	68.829	-2.735	YES	NO	NO
2.	Quercetin	-2.925	-0.229	77.207	-2.735	YES	NO	NO
3.	Catechin	-3.117	-0.283	68.829	-2.735	YES	NO	NO
4.	Kaempferol	-3.04	0.032	74.29	-2.735	YES	NO	NO
5.	Cyanidin Chloride	-2.932	-0.161	71.837	-2.735	YES	NO	NO
6.	Citric acid	-1.423	-0.24	0	-2.735	NO	NO	NO
7.	Caffeic acid	-2.33	0.634	69.407	-2.722	NO	NO	NO
8.	p-coumaric acid	-2.378	1.21	93.494	-2.715	NO	NO	NO
9.	L-Ascorbic acid	-1.556	-0.255	39.154	-2.955	NO	NO	NO
10.	Ferulic acid	-2.817	0.176	93.685	-2.72	NO	NO	NO
11.	Gallic acid	-2.56	-0.081	43.374	-2.735	NO	NO	NO

Table 4.3 continued from previous page

Sr no	Ligand	Water solubility	$CaCO_2$ permeability	Intestinal absorption (human)	Skin Permeability	P-glycoprotein substrate	P-glycoprotein I inhibitor	P-glycoprotein II inhibitor
12.	Salicylic acid	-1.808	1.151	83.887	-2.723	NO	NO	NO
13.	Syringic acid	-2.223	0.495	73.076	-2.735	YES	NO	NO
14.	Camphor	-2.895	1.499	95.965	-2.002	NO	NO	NO
15.	Tartaric acid	-1.667	-0.374	3.709	-2.735	NO	NO	NO
16.	Malic acid	-1.381	-0.395	13.831	-2.735	NO	NO	NO
17.	Myricetin	-2.915	0.095	65.93	-2.735	YES	NO	NO
18.	Rosmarinic acid	-3.059	-0.937	32.516	-2.735	YES	NO	NO
19.	Cyanidin-3-glucoside	-2.904	0.263	29.927	-2.735	YES	NO	NO
20.	Pelargonidin-3-glucoside	-2.759	0.345	32.888	-2.735	YES	NO	NO
21.	Chlorogenic acid	-2.449	-0.84	36.377	-2.735	YES	NO	NO
22.	Cembrene	-7.207	1.458	94.374	-1.675	NO	NO	NO
23.	E-caryophyllene	-5.555	1.423	94.845	-1.58	NO	NO	NO

4.2.2.3 Distribution

After absorption drug is distributed to intestinal and intracellular compartments when it reaches the blood streams. Some of the drugs also bound to the plasma protein in the blood. The binding is reversible so the bound and unbound drug may also exist in dynamic equilibrium. While the unbound drug is considered as pharmacologically active which has the ability to cross the membrane and interact with target sites. The Vd volume of distribution represents the vol. in which a drug needs to distribute to achieve the same conc. as detected in the blood plasma. We can calculate the Vd by division of amount of the drug in the body with plasma conc. Vd.

This gives an insight about how much of the drug is present in extravascular tissue. Since the volume of distributions is affected by lipid solubility, more lipid soluble drugs will have better cell concentration [215].While the drug capacity of dispersion in the body is determined by two important factors: 1) volume of supply at steady state VDss and 2) the blood brain barrier (BBB). If the value of VDs is higher than 0.45 it has good dispersion. When the log BB is higher than 0.33, molecules can pass quickly [217].

According to data available in table 4.4, All these ligands show fraction unbound. For human distribution VDs as per data only 11 compounds (fulfill the criteria i.e. >0.45) with highest among (Quercetin 1.559), (Myricetin 1.317), (Kaempferol 1.274), (Cyanidin-3-glucoside 1.49). These bioactive compounds have shown good distribution into tissues which is considered desirable for most drugs. Whereas, (Citric acid, Caffeic acid, p-coumaric acid, L-Ascorbic acid, Ferulic acid, Gallic acid, Salicylic acid, Syringic acid, Camphor, Tartaric acid, Malic acid, Rosmarinic acid) did not follow the criteria. E-caryophyllene (BBB=0.733), Camphor (BBB=0.612), Cembrene (BBB=0.689) are compounds showing significant potential to act on central nervous system. Most of the flavonoids and acids have shown very low Blood brain barrier permeability and low CNS. These are showing excellent results if the target is not the brain as it will minimize the CNS related side effects.

TABLE 4.4: Distribution parameters for 23 selected ligands from *R. coriaria*

Sr no	**Ligand**	**VDss (human)**	**Fraction unbound (human)**	**BBB permeability**	**CNS permeability**
1.	Epicatechin	1.027	0.235	-1.054	-3.298
2.	Quercetin	1.559	0.206	-1.098	-3.065
3.	Catechin	1.027	0.235	-1.054	-3.298
4.	Kaempferol	1.274	0.178	-0.939	-2.228
5.	Cyanidin Chloride	1.017	0.279	-0.895	-2.865
6.	Citric acid	-0.418	0.51	-1.017	-3.61
7.	Caffeic acid	-1.098	0.529	-0.647	-2.608
8.	p-coumaric acid	-1.151	0.428	-0.225	-2.418
9.	L-Ascorbic acid	0.218	0.825	-0.985	-3.217
10.	Ferulic acid	-1.367	0.343	-0.239	-2.612
11.	Gallic acid	-1.855	0.617	-1.102	-3.74
12.	Salicylic acid	-1.57	0.563	-0.334	-3.21
13.	Syringic acid	-1.443	0.601	-0.191	-2.701
14.	Camphor	0.331	0.459	0.612	-2.158
15.	Tartaric acid	-0.847	0.65	-0.867	-3.987
16.	Malic acid	-0.998	0.652	-0.788	-3.523
17.	Myricetin	1.317	0.238	-1.493	-3.709
18.	Rosmarinic acid	0.393	0.348	-1.378	-3.347
19.	Cyanidin-3-glucoside	1.49	0.298	-1.374	-4.213
20.	Pelargonidin-3-glucoside	1.001	0.284	-1.186	-4.028
21.	Chlorogenic acid	0.581	0.658	-1.407	-3.856
22.	E-caryophyllene	0.652	0.263	0.733	-2.172
23.	Cembrene	0.667	0.107	0.689	-2.206

4.2.2.4 Metabolism

After distribution of the drug in the body, it is metabolized. Drug is converted into polar inactive metabolites so that it is easily eliminated from the body. Lipophilic

drugs can easily pass through biological membranes and reach the target sites easily. This property can hinder their excretion from the body [217].

An enzyme that is involved in detoxification in liver is cytochrome P450. It also plays role in drug metabolism. The pharmacokinetics of drugs is significantly impacted by P450 inhibitors. Therefore, it is important to evaluate if the molecule in question is substrate of CYP2D6/CYP3A4, and can be acted upon by P450. CYP 450 acts on xenobiotics, oxidizing them and allowing their excretion [216].

According to results mentioned in table 4.4, majority of compounds are not substrate or inhibitors of any key CYP enzymes. This is a highly desirable property suggesting that there is a low risk of metabolic interactions with other drugs. While Quercetin, Kaempferol, Myricetin, and Cyanidin Chloride are predicted to inhibit CYP1A2. Cembrene has shown inhibition of CYP2C19 so this requires to take caution in a polypharmacy context.

TABLE 4.5: Metabolism parameters for 23 selected ligands from *R. coriaria* against CYP as an inhibitor

Sr.	**Ligands**	**CYP2D6**	**CYP3A4**	**CYP1A2**	**CYP2C19**	**CYP2C9**	**CYP2D6**	**CYP3A4**
1.	Epicatechin	NO	NO	NO	NO	NO	NO	NO
2.	Quercetin	NO	NO	YES	NO	NO	NO	NO
3.	Catechin	NO	NO	NO	NO	NO	NO	NO
4.	Kaempferol	NO	NO	YES	NO	NO	NO	NO
5.	Cyanidin Chloride	NO	NO	YES	NO	NO	NO	NO
6.	Citric acid	NO	NO	NO	NO	NO	NO	NO
7.	Caffeic acid	NO	NO	NO	NO	NO	NO	NO
8.	p-coumaric acid	NO	NO	NO	NO	NO	NO	NO
9.	L-Ascorbic acid	NO	NO	NO	NO	NO	NO	NO
10.	Ferulic acid	NO	NO	NO	NO	NO	NO	NO
11.	Gallic acid	NO	NO	NO	NO	NO	NO	NO
12.	Salicylic acid	NO	NO	NO	NO	NO	NO	NO
13.	Syringic acid	NO	NO	NO	NO	NO	NO	NO
14.	Camphor	NO	NO	NO	NO	NO	NO	NO
15.	Tartaric acid	NO	NO	NO	NO	NO	NO	NO
16.	Malic acid	NO	NO	NO	NO	NO	NO	NO
17.	Myricetin	NO	NO	YES	NO	NO	NO	NO

Table 4.5 continued from previous page

Sr.	Ligands	CYP2D6	CYP3A4	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
18.	Rosmarinic acid	NO	NO	NO	NO	NO	NO	NO
19.	Cyanidin - 3 - glucoside	NO	NO	NO	NO	NO	NO	NO
20.	Pelargonidin - 3 - glucoside	NO	NO	NO	NO	NO	NO	NO
21.	Chlorogenic acid	NO	NO	NO	NO	NO	NO	NO
22.	E-caryophyllene	NO	NO	NO	NO	NO	NO	NO
23.	Cembrene	NO	NO	NO	YES	NO	NO	NO

4.2.2.5 Excretion

Drugs after metabolism are excreted by two main ways via renal excretion and liver, some of the drugs can also be secreted by saliva, tears and sweat in negligible amount [214]. Renal OCT2 substrate helps and acts as transporter that helps in eliminating the drugs and other compounds from the body. While the total clearance indicates the hepatic clearance by metabolizing it and renal clearance is indication of excreted drug [218].

According to table 4.6, Cembrene (1.48) and E-caryophyllene (1.088), Tartaric acid (0.887) and Citric acid (0.895) have fast clearance from the body. Camphor (0.109), Epicatechin/Catechin (0.183), Rosmarinic acid (0.25), Chlorogenic acid (0.307) can stay in body for longer period of time but have risk of accumulation. All other compounds fall in middle range of (0.4-0.7).

TABLE 4.6: Excretion parameters for 23 selected ligands from *R. coriaria*

Sr no.	Ligand	Total Clearance	Renal OCT2 Substrate
1.	Epicatechin	0.183	NO
2.	Quercetin	0.407	NO
3.	Catechin	0.183	NO
4.	Kaempferol	0.477	NO

Table 4.6 continued from previous page

Sr no.	Ligand	Total Clearance	Renal OCT2 Substrate
5.	Cyanidin Chloride	0.525	NO
6.	Citric acid	0.895	NO
7.	Caffeic acid	0.508	NO
8.	p-coumaric acid	0.622	NO
9.	L-Ascorbic acid	0.631	NO
10.	Ferulic acid	0.623	NO
11.	Gallic acid	0.518	NO
12.	Salicylic acid	0.607	NO
13.	Syringic acid	0.646	NO
14.	Camphor	0.109	NO
15.	Tartaric acid	0.887	NO
16.	Malic acid	0.81	NO
17.	Myricetin	0.422	NO
18.	Rosmarinic acid	0.25	NO
19.	Cyanidin-3-glucoside	0.548	NO
20.	Pelargonidin-3-glucoside	0.591	NO
21.	Chlorogenic acid	0.307	NO
22.	E-caryophyllene	1.088	NO
23.	Cembrene	1.48	NO

4.2.2.6 Toxicity

LD50 is the toxic dose that is measured in mg/kg of weight. LD refers to the lethal dose indicating the amount of specific compound that can cause death of 50% of the experimental population. In case of *T. pyriformis* if value is >-0.5 than it is considered as highly toxic [219].

By using *Salmonella typhimurium*, AMES test is employed for analyzing the mutagenicity of a compound. When the bacterial cells are exposed to any mutagenic materials, they will undergo certain mutations.

This environment will help bacteria to grow without histidine. Positive results indicate that compound is mutagenic and can cause cancer. hERG I and hERG II indicate the cardiotoxicity by causing lethal arrythmias of ventricles after inhibiting potassium channels.

Hepatoxicity is an important parameter counted for any drug so it should have negative in results. The oral rat toxicity (dose 0.341 to 2.674) studies the impact of drug on body after repeated ingestion, skin inhalation and inhalation for certain period of time. This parameter helps to evaluate the MRTD for clinical trials on humans.

MRTD actually estimates a threshold when substance becomes toxic to humans and is important for determination of dose during clinical trials [220].

Minnow toxicology test helps to evaluate the dose which causes 50% death of research animals (fathead minnows). Substances have acute toxicity when they have low LC50 (less than 0.5 mM or Log LC50 less than -0.3). Skin sensitivity will show about the allergic reactions after coming in contact [221].

According to table 4.7, as the AMES toxicity is predicting the mutagenicity of any compound while all compounds show negative result, is an excellent indicator.

In maximum tolerated dose in humans, lower values indicate the potential toxicity so most of the compounds are safe except chlorogenic acid (-0.134) which has shown very low predicted dose and considered not a good characteristic.

hERG predicts cardiotoxicity where all compounds have shown negative results and considered safe except Cyanidin-3-glucoside and Pelargonidin-3-glucoside may pose cardiac toxicity risk due to hERG II inhibition. Hepatotoxicity results were also observed negative.

E-caryophyllene, Camphor, and Cembrene are predicted to cause skin sensitization. For minnow toxicity higher values indicate least toxic patterns of compounds.

Most of the compounds are safe except Cembrene (-0.448) which is predicted to be highly toxic.

TABLE 4.7: Toxicity parameters for 23 selected ligands from *R. coriaria*.

Sr	Ligands	AMES Toxicity	Max. tolerated dose (human)	hERG I Inhibitor	hERG II Inhibitor	Oral Rat Acute Toxicity (LD50) Mol/kg	Oral Rat Chronic Toxicity (LOAEL)	Hepato Toxicity	Skin Sensitisation	T . Pyriformis Toxicity	Minnow Toxicity
1.	Epicatechin	NO	0.438	NO	NO	2.428	2.5	NO	NO	0.347	3.585
2.	Quercetin	NO	0.499	NO	NO	2.471	2.612	NO	NO	0.288	3.721
3.	Catechin	NO	0.438	NO	NO	2.48	2.5	NO	NO	0.347	3.585
4.	Kaempferol	NO	0.531	NO	NO	2.449	2.505	NO	NO	0.312	2.885
5.	Cyanidin Chloride	NO	0.492	NO	NO	2.456	2.703	NO	NO	0.289	3.087
6.	Citric acid	NO	0.749	NO	NO	2.148	3.698	NO	NO	0.285	4.251
7.	Caffeic acid	NO	1.145	NO	NO	2.383	2.092	NO	NO	0.293	2.246
8.	p-coumaric acid	NO	1.111	NO	NO	2.155	2.534	NO	NO	0.319	1.607
9.	L-Ascorbic acid	NO	1.598	NO	NO	1.063	3.186	NO	NO	0.285	4.386
10.	Ferulic acid	NO	1.082	NO	NO	2.282	2.065	NO	NO	0.271	1.825
11.	Gallic acid	NO	0.7	NO	NO	2.218	3.06	NO	NO	0.285	3.188
12.	Salicylic acid	NO	0.61	NO	NO	2.282	2.483	NO	NO	0.263	1.812
13.	Syringic acid	NO	1.374	NO	NO	2.157	2.415	NO	NO	0.281	2.554

Table 4.7 continued from previous page

Sr	Ligands	AMES Toxicity	Max. tolerated dose	hERG I Inhibitor	hERG II Inhibitor	Oral Rat Acute Toxicity	Oral Rat Chronic Toxicity	Hepato Toxicity	Skin Sensitisation	T . Pyriformis Toxicity	Minnow Toxicity
14.	Camphor	NO	0.473	NO	NO	1.653	1.981	NO	YES	0.233	1.458
15.	Tartaric acid	NO	1.212	NO	NO	1.744	3.769	NO	NO	0.285	3.965
16.	Malic acid	NO	1.212	NO	NO	1.818	3.104	NO	NO	0.285	3.348
17.	Myricetin	NO	0.51	NO	NO	2.4973	2.718	NO	NO	0.286	5.02
18.	Rosmarinic acid	NO	0.152	NO	NO	2.811	2.907	NO	NO	0.302	2.698
19.	Cyanidin - 3 - glucoside	NO	0.562	NO	YES	2.547	4.362	NO	NO	0.285	6.937
20.	Pelargonidin - 3 - glucoside	NO	0.537	NO	YES	2.566	4.659	NO	NO	0.285	5.708
21.	Chlorogenic acid	NO	-0.134	NO	NO	1.973	2.982	NO	NO	0.285	5.741
22.	E - caryophyllene	NO	0.351	NO	NO	1.617	1.416	NO	YES	1.401	0.504
23.	Cembrene	NO	0.269	NO	NO	1.512	1.244	NO	YES	2.031	-0.448

4.3 Molecular Docking

RMSD (Root Mean Square Deviation) values are used to assess the accuracy of molecular docking results by comparing the docked pose of a ligand to a reference pose, such as the experimentally determined pose in PyRx tool. A more precise docking result is usually represented by a lower RMSD value; values below 2 Åare often seen as a good indicator of a reliable docking conclusion [222]. RSMD (Root Mean Square Deviation) are given in PyRx results of docking to access the accuracy of molecular docking results. A lower RSMD value generally gives more accurate results, usually below 2 Åis a good indication while the 0 value is ideally selected for results [223].

For molecular docking 23 compounds were selected after Lipinski rule and ADMET properties for performing analysis of interaction between ligands and protein.

On the basis of analysis, the 2D structure shows the different types of bonding including (hydrogen bond, hydrophobic bond and electrostatic bond) between Fusion protein and ligands. Among all ligands, Cyanidin-3-glucoside have shown highest no. of interactions by showing 11 bonds (8 hydrogen, 1 hydrophobic and 1 electrostatic bonds) with highest binding affinity of -7.9 kcal/mol.

Myricetin, Epicatechin, Pelargonidin-3-glucoside, Quercetin, Catechin, Chlorogenic acid, Kaempferol, Cyanidin Chloride, Rosmarinic acid have shown binding affinities ranging from (-7.8 o -7.1) shown in table 4.8. Whereas Myricetin has shown (6 hydrogen bonds, and 2 hydrophobic bonds. Epicatechin (4 hydrogen bonds with 1 electrostatic bond), Pelargonidin-3-glucoside (5 hydrogen bonds, 1 hydrophobic and 1 electrostatic bond), Quercetin (7 hydrogen bonds with 2 hydrophobic bonds), Catechin (1 hydrogen and electrostatic bond with 2 hydrophobic bonds), Chlorogenic acid (4 hydrogen bonds, 3 hydrophobic bonds and only 1 electrostatic bond), Kaempferol (3 hydrogen bonds, 3 hydrophobic bonds with 2 electrostatic bonds), Cyanidin Chloride (6 hydrogen bond and 2 hydrophobic bonds), Rosmarinic acid (5 hydrogen bond and 2 hydrophobic bonds) are seen and mentioned in table 4.9 below.

Malic acid has shown lowest binding affinity with -4.9 among all compounds with only 1 hydrogen bond while no hydrophobic and electrostatic bonds are seen.

While on the other hand smaller phenolics including Citric acid, Malic acid, and Ferulic acid showed fewer interactions (2 to 5 bonds), reflecting their lower binding affinities.

Whereas, Cembrene and E-caryophyllene showed no interactions, suggesting poor binding affinity and low potential as effective ligands.

TABLE 4.8: 23 Selected ligands with their Binding affinities and 3D structures of docked complex of ligands with protein.

Sr no.	**Ligand Name**	**Binding Affinity**	**RMS Value**	**Structure of 3D Complex**
1.	Cyanidin - 3 - glucoside	-7.9	0	
2.	Myricetin	-7.8	0	
3.	Epicatechin	-7.7	0	
4.	Pelargonidin-3-glucoside	-7.7	0	

Table 4.8 continued from previous page

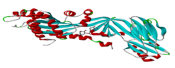
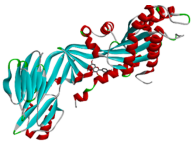
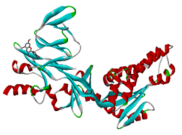
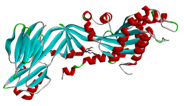
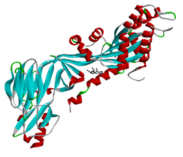
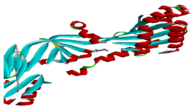
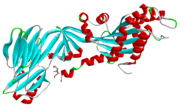
Sr no.	Ligand Name	Binding Affinity	RMS Value	Structure of 3D Complex
5.	Quercetin	-7.7	0	
6.	Catechin	-7.4	0	
7.	Chlorogenic acid	-7.4	0	
8.	Kaempferol	-7.4	0	
9.	Cyanidin Chloride	-7.3	0	
10.	Rosmarinic acid	-7.1	0	
11.	Cembrene	-6.5	0	
12.	E-caryophyllene	-6.4	0	
13.	Citric acid	-5.8	0	

Table 4.8 continued from previous page

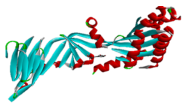
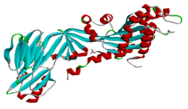
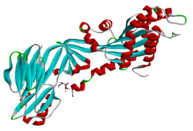
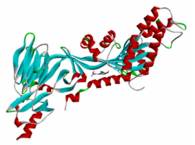
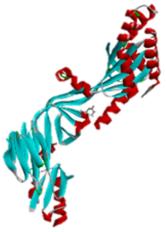
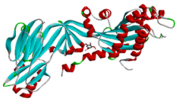
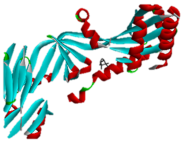
Sr no.	Ligand Name	Binding Affinity	RMS Value	Structure of 3D Complex
14.	Caffeic acid	-5.8	0	
15.	p-coumaric acid	-5.8	0	
16.	L-Ascorbic acid	-5.7	0	
17.	Ferulic acid	-5.6	0	
18.	Gallic acid	-5.5	0	
19.	Salicylic acid	-5.3	0	
20.	Syringic acid	-5.3	0	
21.	Camphor	-5.1	0	

Table 4.8 continued from previous page

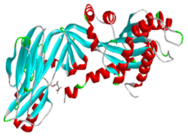
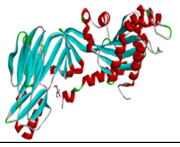
Sr no.	Ligand Name	Binding Affinity	RMS Value	Structure of 3D Complex
22.	Tartaric acid	-5	0	
23.	Malic acid	-4.9	0	

TABLE 4.9: Binding Interaction of selected Ligands and Fusion Protein

Sr no.	Ligands	Binding affinities	RMS	Types of interaction	Amino acid residues with bond distance
1	Cyanidin-3-glucoside	-7.9	0	01. Hydrogen bond	LYS287;3.02
					GLN307;2.13
					TYR319;1.89
					GLU305;2.62
					ALA288;2.86
					GLN307;2.30
					TYR320;1.96
					CYS326;2.59
				02. Hydrophobic bond	GLU323;3.49
				03. Electrostatic bond	GLU232;4.84
2	Myricetin	-7.8	0	01.Hydrogen bond	SER293;3.06
					GLU294;3.06
					TYR23;2.02
					GLU294;2.51
					GLY406;3.33
					CYS407;3.79
				02. Hydrophobic bond	CYS407;4.38
3	Epicatechin	-7.7	0	01.Hydrogen bond	GLY106;2.87
					GLN307;2.98
					TYR319;2.86
					ASN457;2.52
				02.Electrostatic bond	GLU305;3.88

Table 4.9 continued from previous page

Sr no.	Ligands	Binding affinities	RMS	Types of interaction	Amino acid residues with bond distance
4	Pelargonidin-3-glucoside	-7.7	0	01.Hydrogen bond	LYS287;3.00
					GLN307;2.24
					TYR319;1.77
					GLU323;2.34
					CYS326;2.64
					LYS287;3.67
				02.Hydrophobic bond	GLU232;3.51
				03.Electrostatic bond	GLU323;4.43
5	Quercetin	-7.7	0	01.Hydrogen bond	SER291;2.71
					SER293;3.09
					GLU294;3.07
					SER444;2.70
					TYR23;2.01
					GLY406;3.34
					CYS407;3.85
				02.Hydrophobic bond	CYS407; 4.43
					PRO441;3.94
6	Catechin	-7.4	0	01.Hydrogen bond	LYS324; 2.30
				02.Hydrophobic bond	PHE256;4.71
					ALA117;5.09
				03.Electrostatic bond	LYS254; 4.76
7	Chlorogenic acid	-7.4	0	01.Hydrogen bond	THR114; 2.83
					PHE334; 1.89
					ARG253; 3.00
					GLY255; 3.66
				02.Hydrophobic bond	LYS254;
					3.16919
					CYS335;4.23293
					ARG40;
					5.06973
				03.Electrostatic bond	LYS254;3.99
8	Kaempferol	-7.4	0	01.Hydrogen bond	THR114;2.57
					LYS324;2.26
					LYS254;4.12

Table 4.9 continued from previous page

Sr no.	Ligands	Binding affinities	RMS	Types of interaction	Amino acid residues with bond distance
				02.Hydrophobic bond	PHE256;4.56
					LYS254;4.11
					ALA117;4.49
				03.Electrostatic bond	ASP336;3.39
					LYS254;4.12
9	Cyanidin Chloride	-7.3	0	01.Hydrogen bond	SER291;2.79
					GLU25;2.78
					SER444;2.51
					GLU294;2.04
					GLY406;3.28
					CYS407;3.83
				02.Hydrophobic bond	CYS407;4.44
					PRO441;3.96
10	Rosmarinic acid	-7.1	0	01.Hydrogen bond	PRO215;2.42
					PRO215;1.98
					THR214;2.81
					PRO215;2.28
					PRO215;3.50
				02.Hydrophobic bond	PHE;256;4.47
					ALA117;5.15
11	Citric acid	-5.8	0	01. Hydrogen bond	GLN307;2.99
					TYR319;3.06
					GLU305;2.16
					GLU305;2.30
12	Caffeic acid	-5.8	0	01.Hydrogen bond	PRO215;2.02
					GLY255;2.96
					ASP325;3.60
				02.Hydrophobic bond	ALA117;5.38
					LYS254;5.13
13	p-_coumaric acid	-5.8	0	01.Hydrogen bond	THR114;3.17
					ASP336;1.94
					THR214;2.29
					PRO215;3.59
				02.Hydrophobic bond	PHE256;4.05
					ALA117;4.99

Table 4.9 continued from previous page

Sr no.	Ligands	Binding affinities	RMS	Types of interaction	Amino acid residues with bond distance
14	L-Ascorbic acid	-5.7	0	01.Hydrogen bond	GLN307;3.31
					TYR319;2.75
					TYR319;2.84
					ASN457;3.13
					GLU305;2.03
15	Ferulic acid	-5.6	0	01.Hydrogen bond	THR114;2.91
					THR214;2.08
				02.Hydrophobic bond	PRO215;5.14
16	Gallic acid	-5.5	0	01.Hydrogen bond	THR114;3.11
					ASP336;3.27
					THR114;2.08
					THR114;3.47
				02.Hydrophobic bond	ALA113;4.86
					LYS254;5.30
				03.Electrostatic bond	ASP336;3.33
17	Salicylic acid	-5.3	0	01.Hydrogen bond	SER291;2.76
					SER291;3.05
					SER443;2.95
					CYS292;1.86
					CYS407;3.94
				02.Hydrophobic bond	CYS407;3.66
					PRO441;4.02
18	Syringic acid	-5.3	0	01.Hydrogen bond	THR114;2.96
					ARG253;2.07
				02.Hydrophobic bond	LYS254;4.72
					LYS254;5.15
					PHE256;4.25
					ALA117;5.45
					PHE256;4.25
					ALA117;5.45
19	Camphor	-5.1	0	01.Hydrogen bond	THR114;3.06
				02.Hydrophobic bond	ALA117;5.23
					PHE256;5.01
20	Tartaric acid	-5	0	01.Hydrogen bond	GLN307;2.35
					TYR319;2.09

Table 4.9 continued from previous page

Sr no.	Ligands	Binding affinities	RMS	Types of interaction	Amino acid residues with bond distance
					ARG304;2.77 GLU305;2.60 GLU305;2.60 ASN457;2.54 ASN457;2.54 GLU305;2.67
21	Malic acid	-4.9	0	01.Hydrogen bond	TYR319;3.188
22	Cembrene	-6.5	0	01. Hydrogen bond 02. Hydrophobic bond 03. Electrostatic bond	 NO BOND-INGS
23	E-caryophyllene	-6.4	0	01. Hydrogen bond 02. Hydrophobic bond 03. Electrostatic bond	 NO BOND-INGS

4.3.1 Analysis and Visualization of Protein Ligand Interaction

After molecular docking by PyRx the results were analyzed by selecting the best pose on the basis of the highest binding affinities in discovery studio.

The best pose was saved in PDB format and opened in Discovery Studio along with cleaned protein to analyze the interaction of ligand with the protein by identifying hydrogen bonds, hydrophobic interactions along with electrostatic interactions. Bond size was also noted in hydrogen bonding.

These interactions helped us to find the mechanism of action of the ligand and helps in further optimization of their structure.

The detail of all docked compounds in image form is given below.

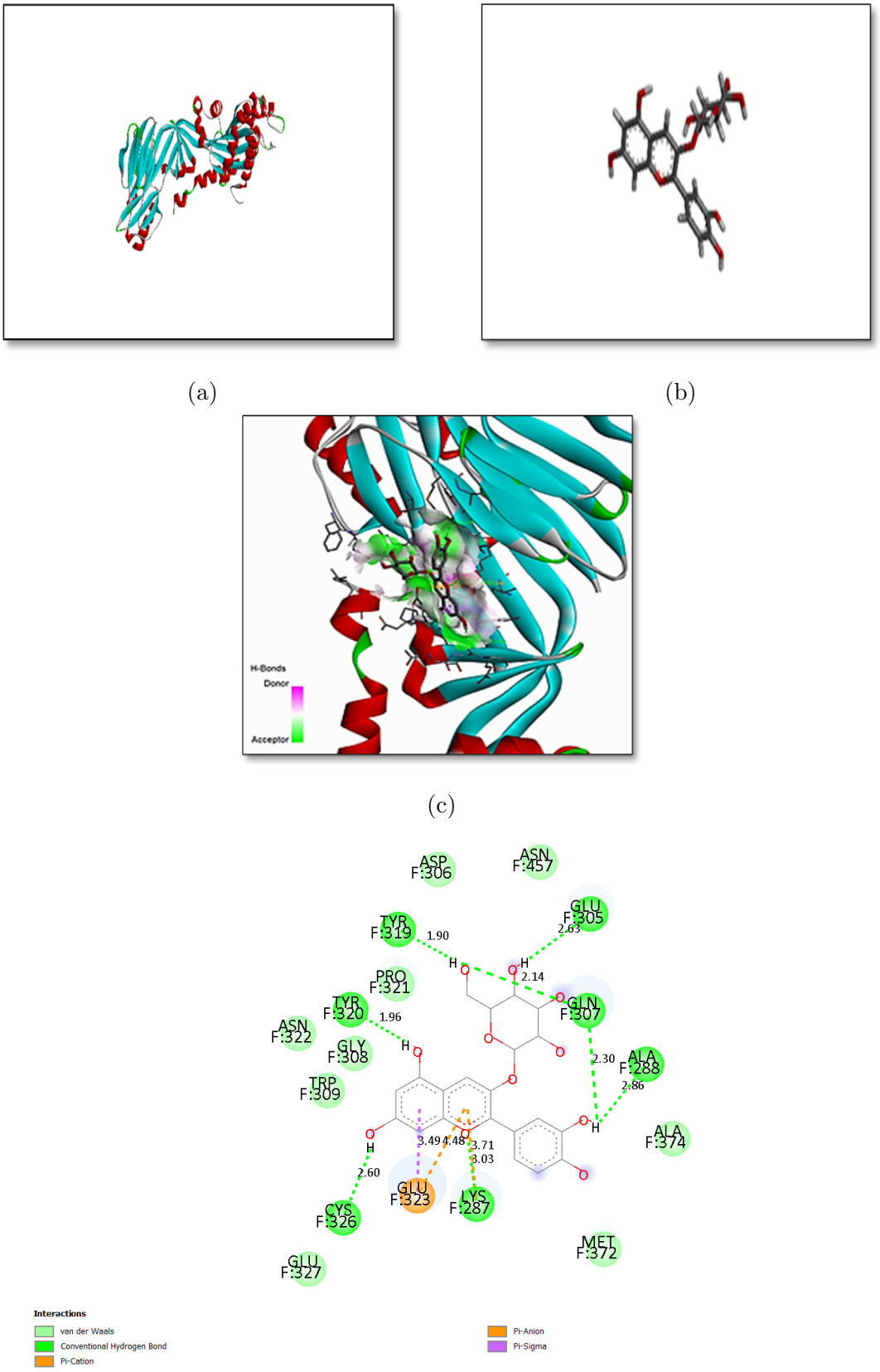


FIGURE 4.3: Molecular Docking analysis of Fusion protein and Cyanidin-3-glucoside. (a) 3D structure of hMPV Fusion protein (b) 3D structure of Cyanidin-3-glucoside (c) SAS view of hMPV Protein and Cyanidin-3-glucoside complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

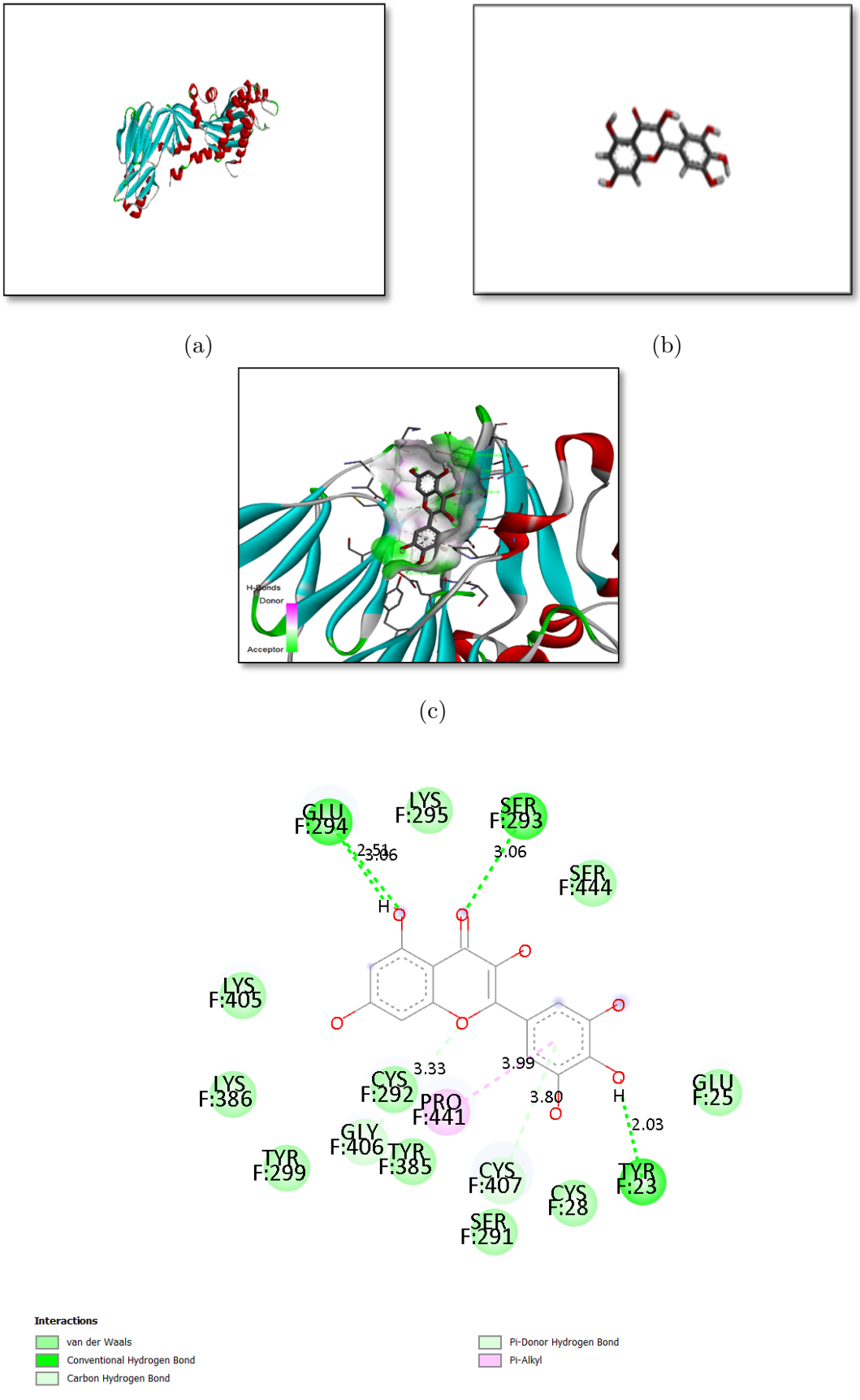


FIGURE 4.4: Molecular Docking analysis of Fusion protein and Myricetin (a)3D structure of hMPV Fusion protein (b) 3D structure of Myricetin c) SAS view of hMPV Protein and Myricetin complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

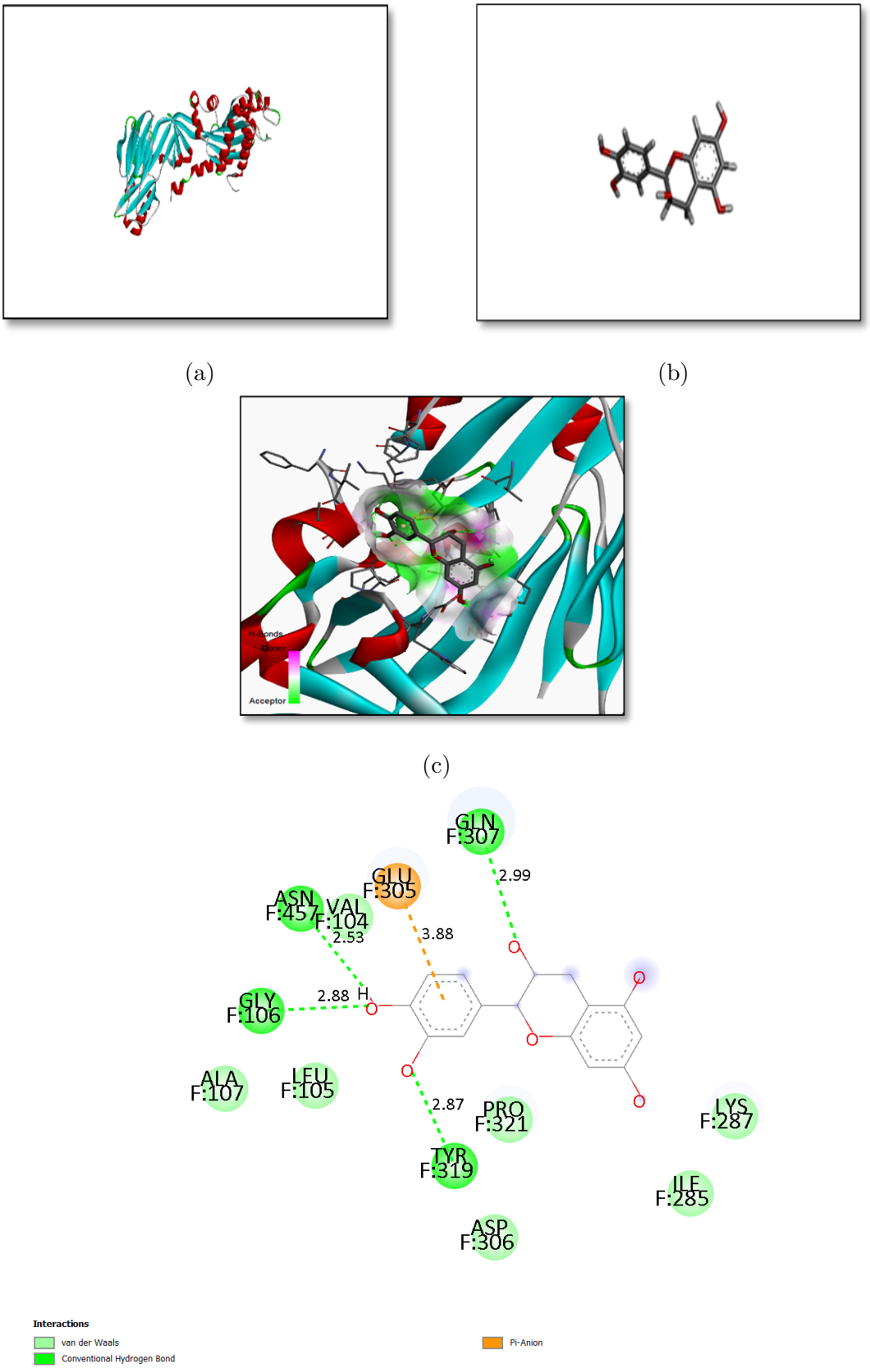


FIGURE 4.5: Molecular Docking analysis of Fusion protein and Epicatechin a) 3D structure of hMPV Fusion protein (b) 3D structure of Epicatechin (c) SAS view of Protein and Epicatechin complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

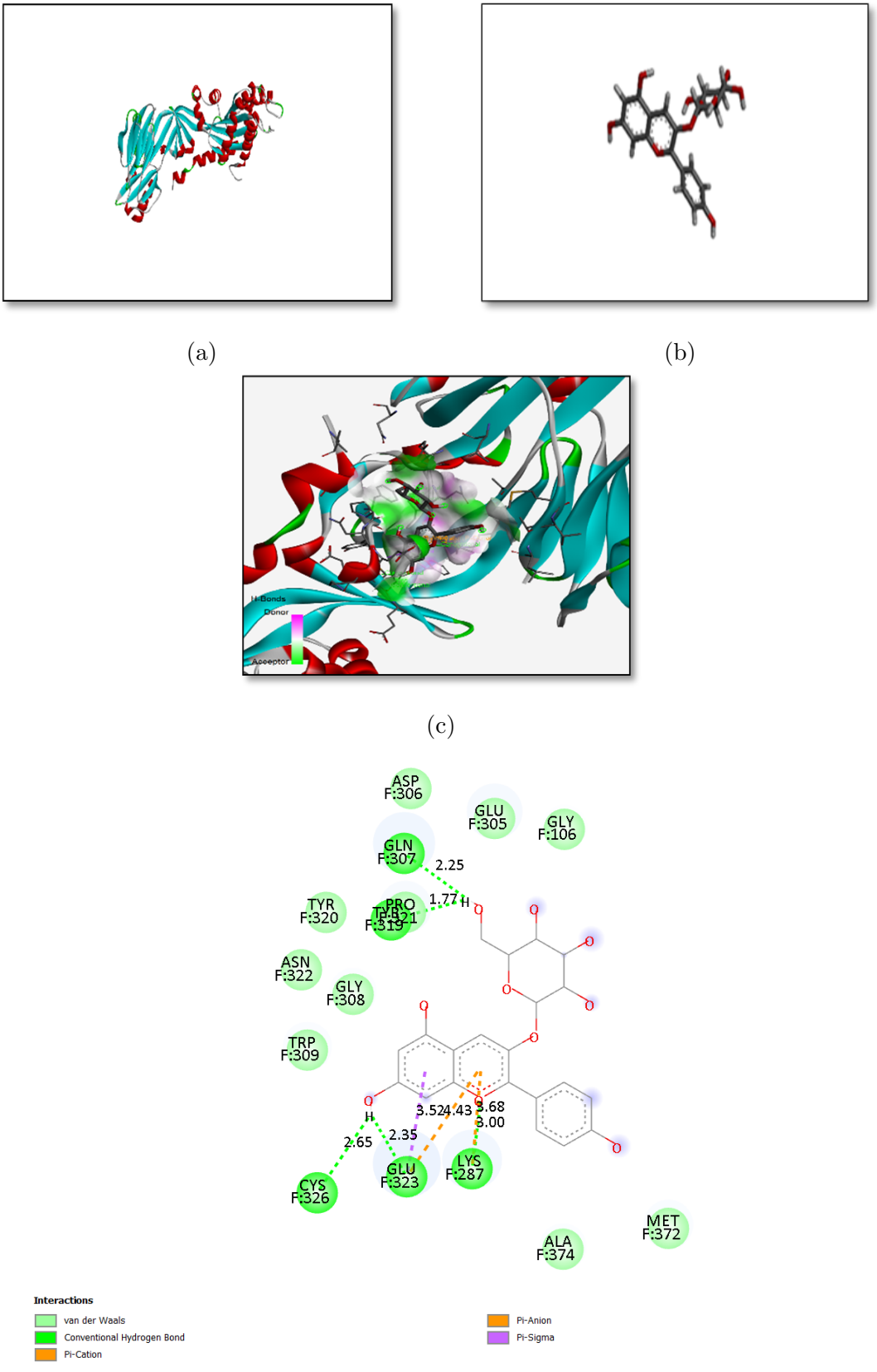


FIGURE 4.6: Molecular Docking analysis of Fusion protein and Pelargonidin-3-glucoside (a) 3D structure of hMPV Fusion protein (b) 3D structure of Pelargonidin-3-glucoside (c) SAS view of hMPV Protein and Pelargonidin-3-glucoside complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

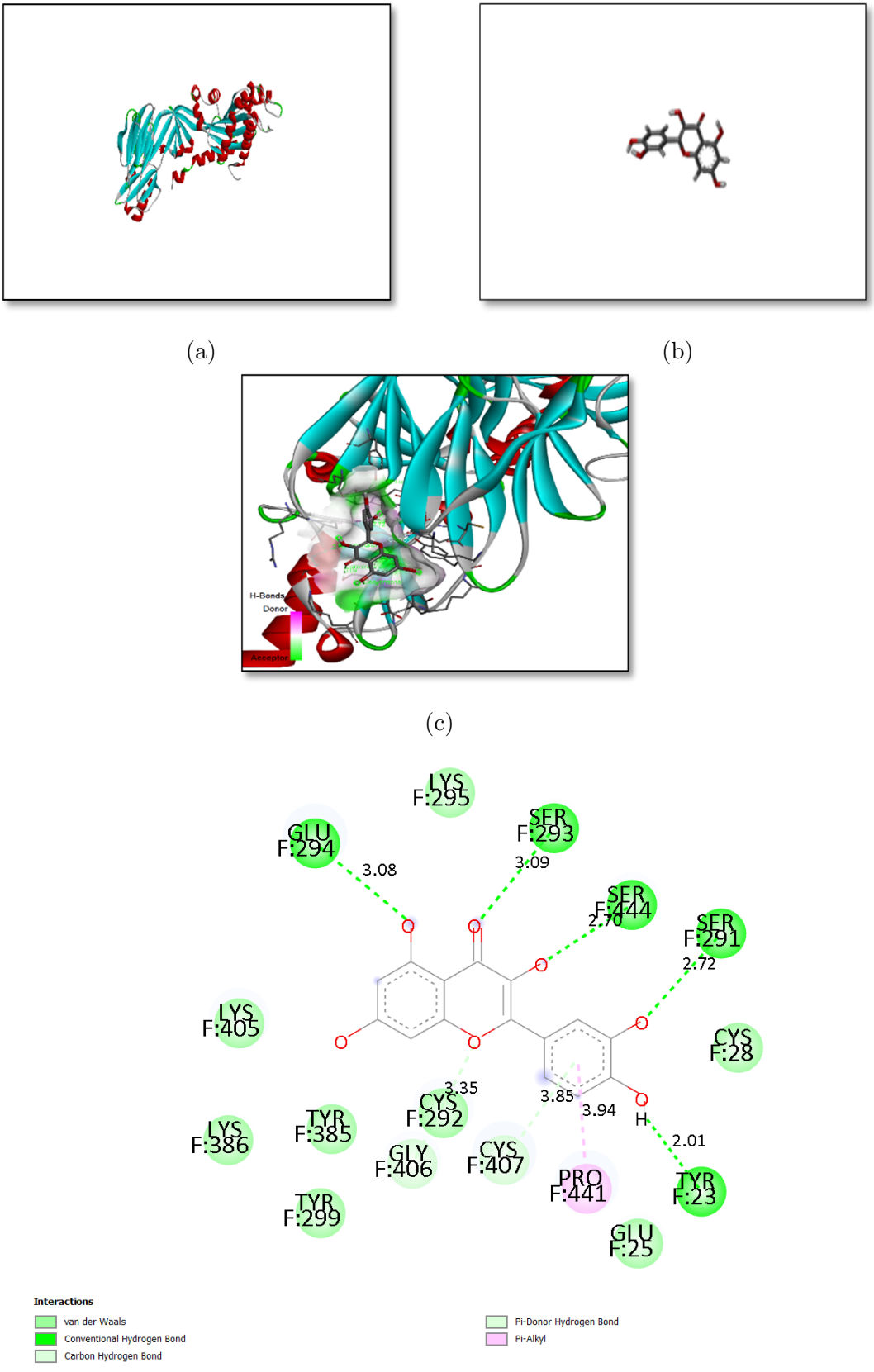


FIGURE 4.7: Molecular Docking analysis of Fusion protein and Quercetin (a) 3D structure of hMPV Fusion protein (b) 3D structure of Quercetin (c) SAS view of hMPV Protein and Quercetin complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

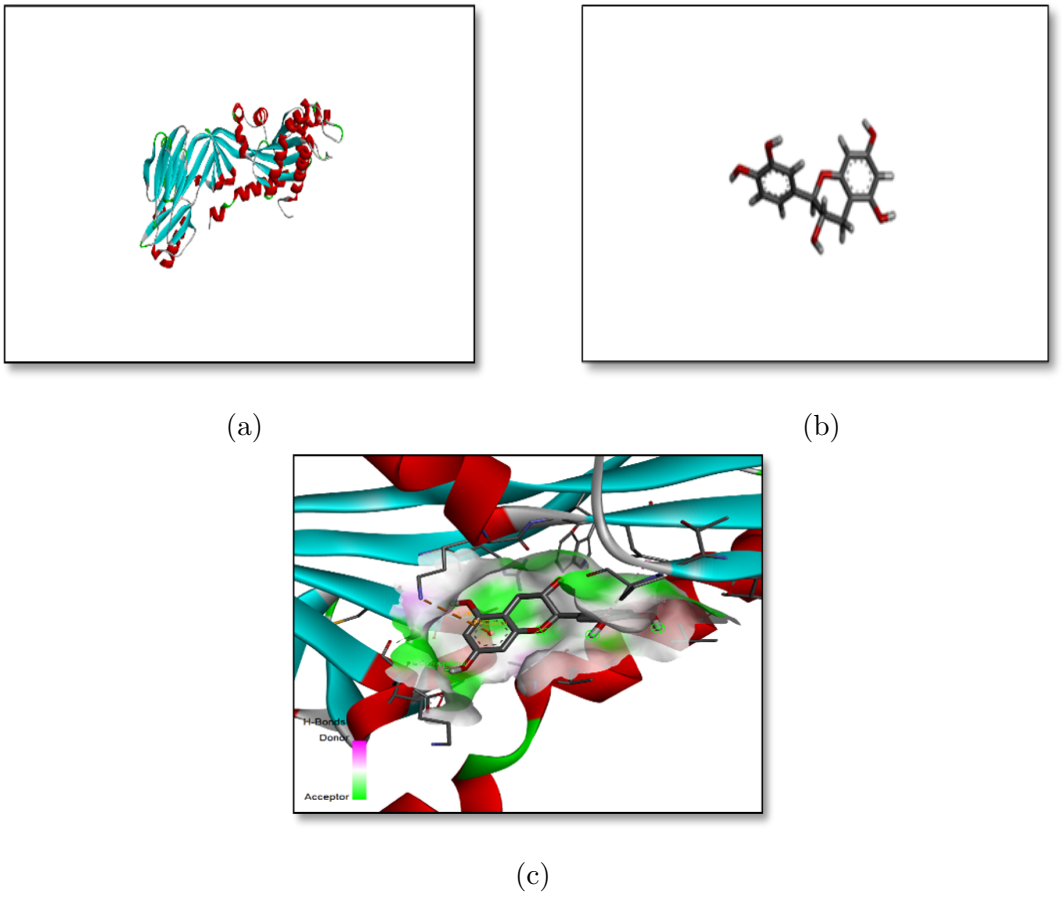


FIGURE 4.8: Molecular Docking analysis of Fusion protein and Catechin (a)3D structure of hMPV Fusion protein (b) 3D structure of Catechin (c) SAS view of hMPV Protein and Catechin complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

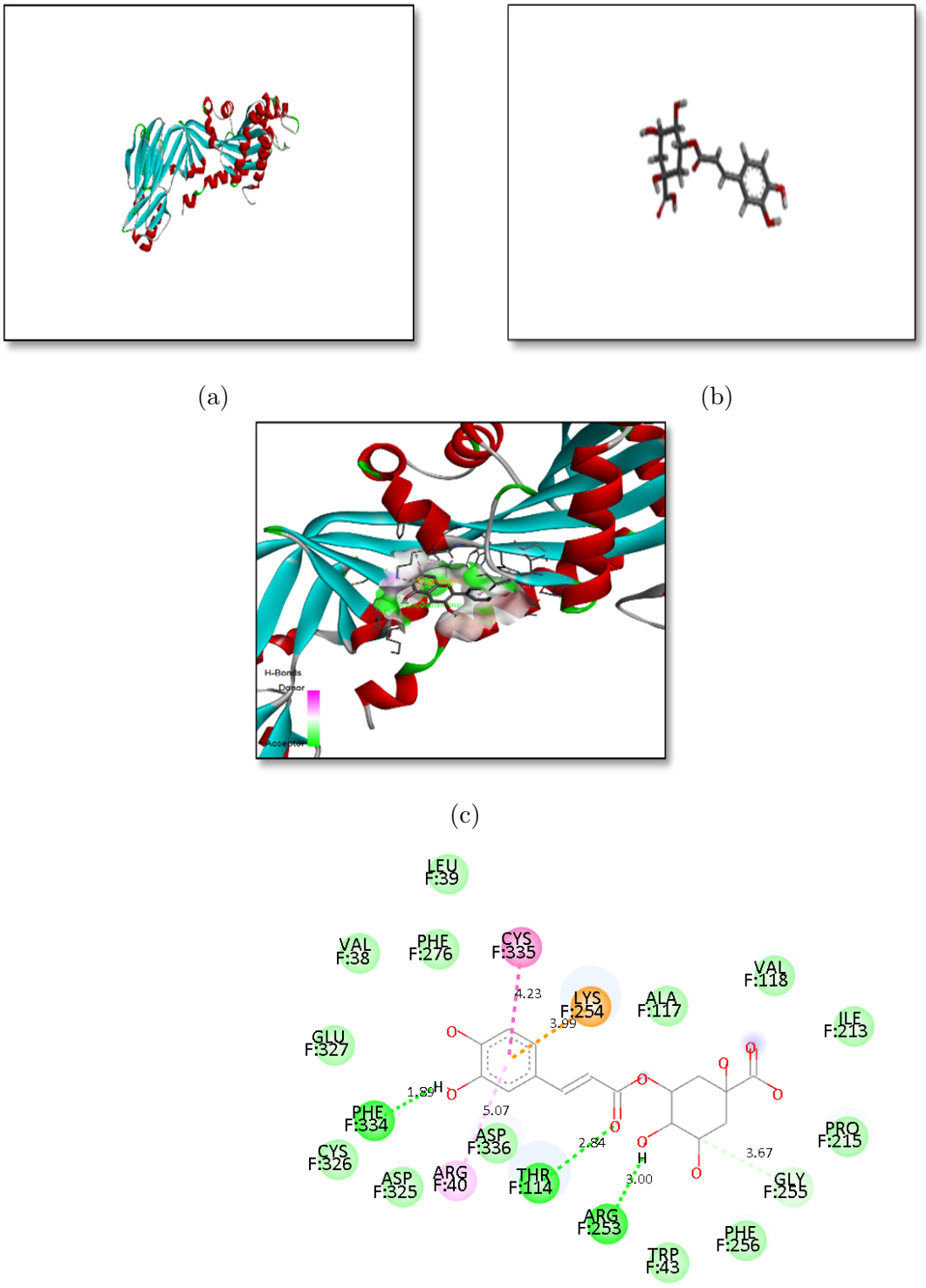


FIGURE 4.9: Molecular Docking analysis of Fusion protein and Chlorogenic acid (a) 3D structure of hMPV Fusion protein (b) 3D structure of Chlorogenic acid (c) SAS view of hMPV Protein and Chlorogenic acid complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

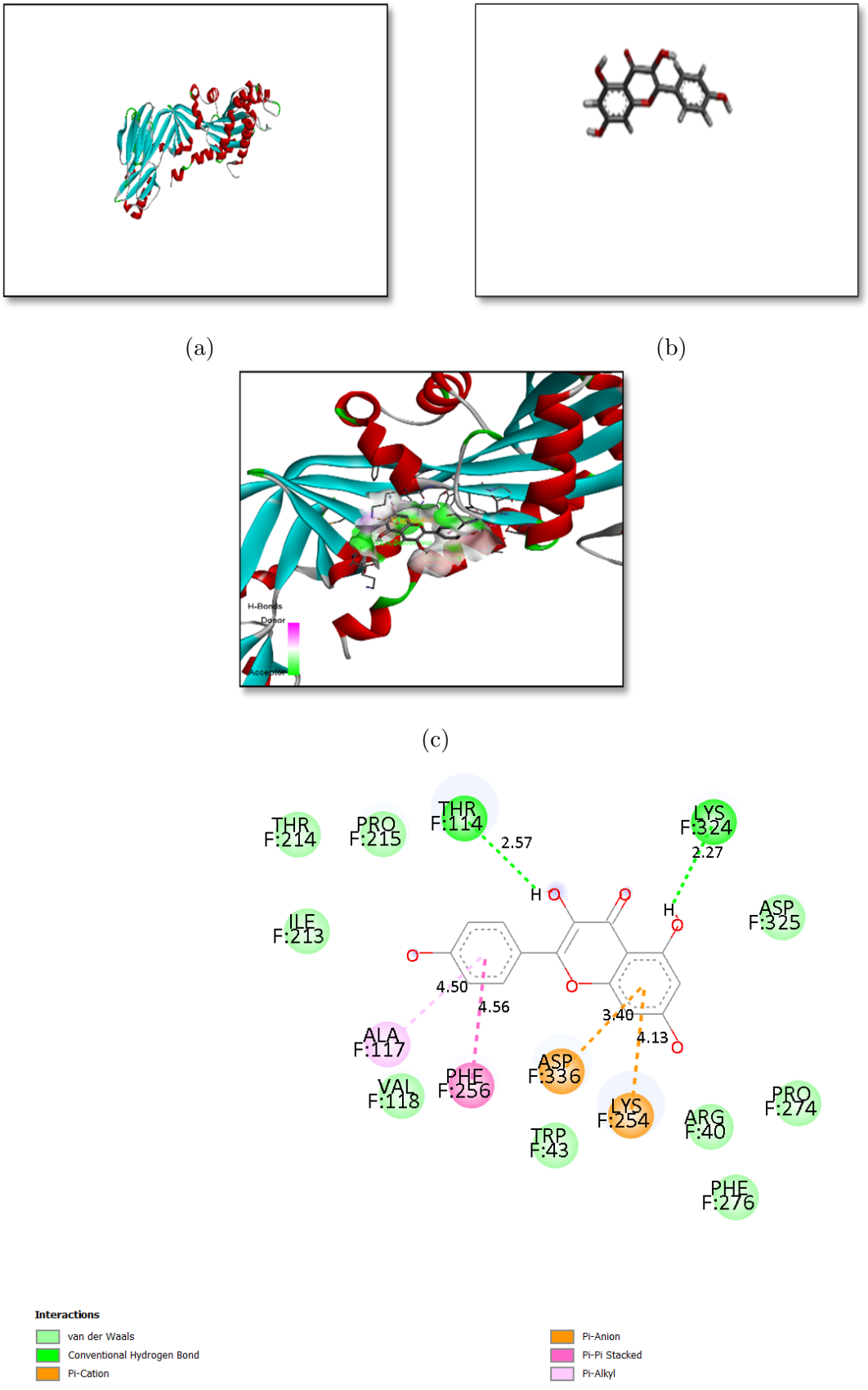
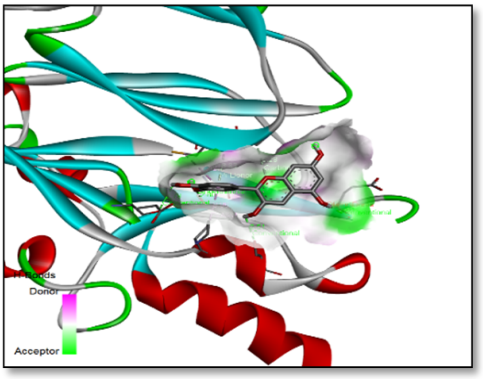


FIGURE 4.10: Analysis of Molecular Docking of Fusion protein and Kaempferol. (a) 3D structure of hMPV Fusion protein (b) 3D structure of Kaempferol (c) SAS view of hMPV Protein and Kaempferol complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

(a) (b)



(c)

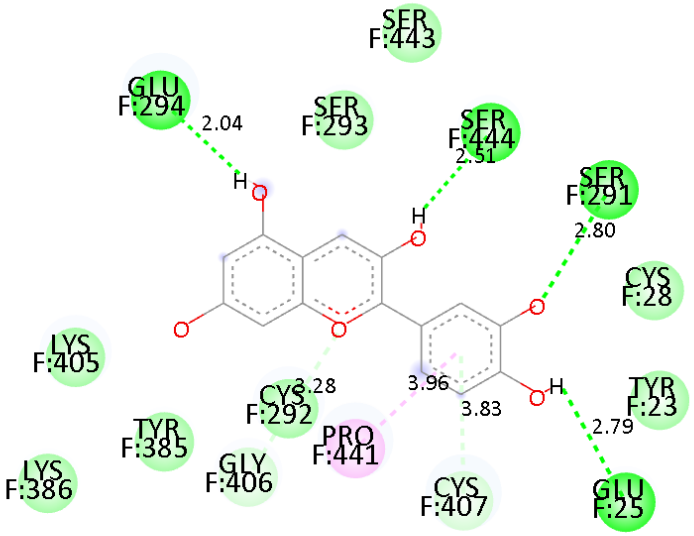


FIGURE 4.11: Analysis of Molecular Docking of Fusion protein and Cyanidin chloride (a) 3D structure of hMPV Fusion protein (b) 3D structure of Cyanidin chloride (c) SAS view of hMPV Protein and Cyanidin chloride complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

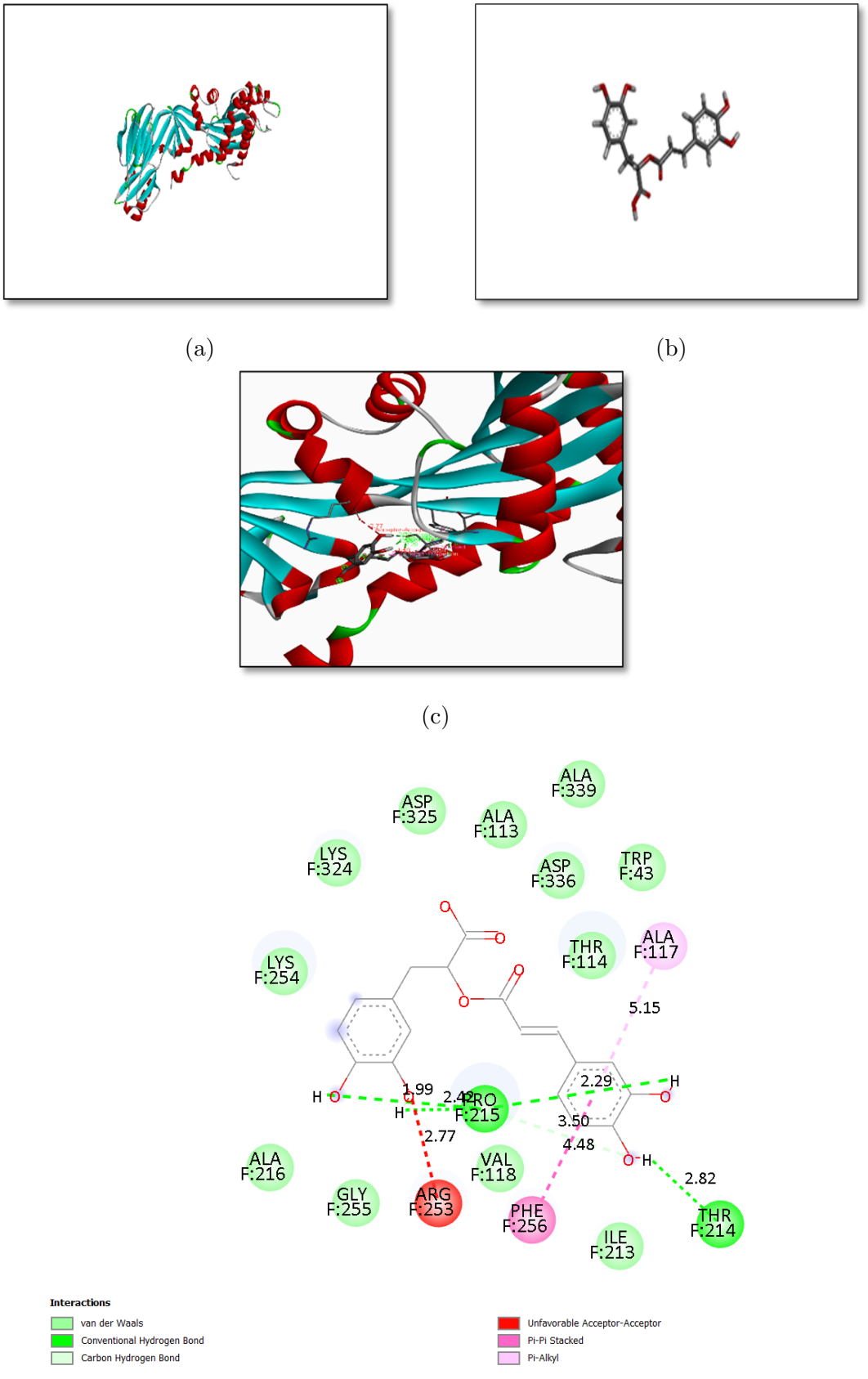
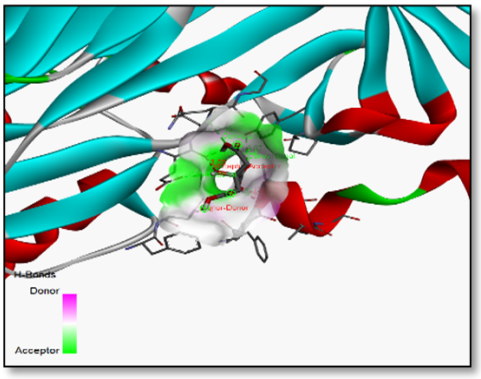


FIGURE 4.12: Molecular Docking analysis of Fusion protein and Rosmarinic acid. (a) 3D structure of hMPV Fusion protein (b) 3D structure of Rosmarinic acid (c) SAS view of hMPV Protein and Rosmarinic acid complex (d) 2D interaction of active site of Ligand and protein amino acids residues of Fusion protein.

(a) (b)



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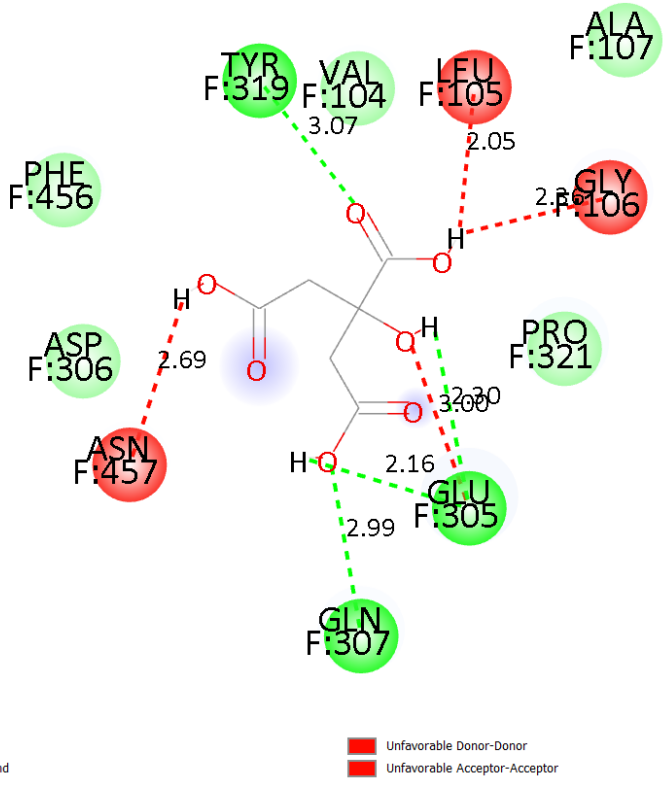
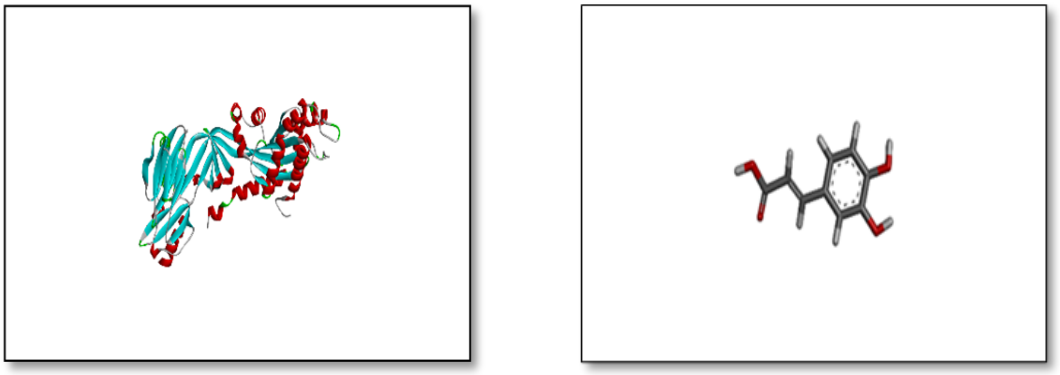


FIGURE 4.13: Molecular Docking analysis of Fusion protein and Citric acid (a) 3D structure of hMPV Fusion protein (b) 3D structure of Citric acid (c) SAS view of hMPV Protein and Citric acid complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.



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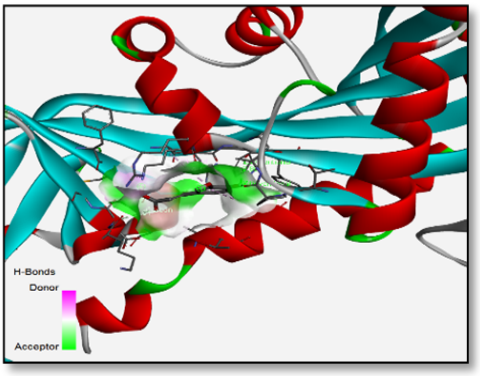

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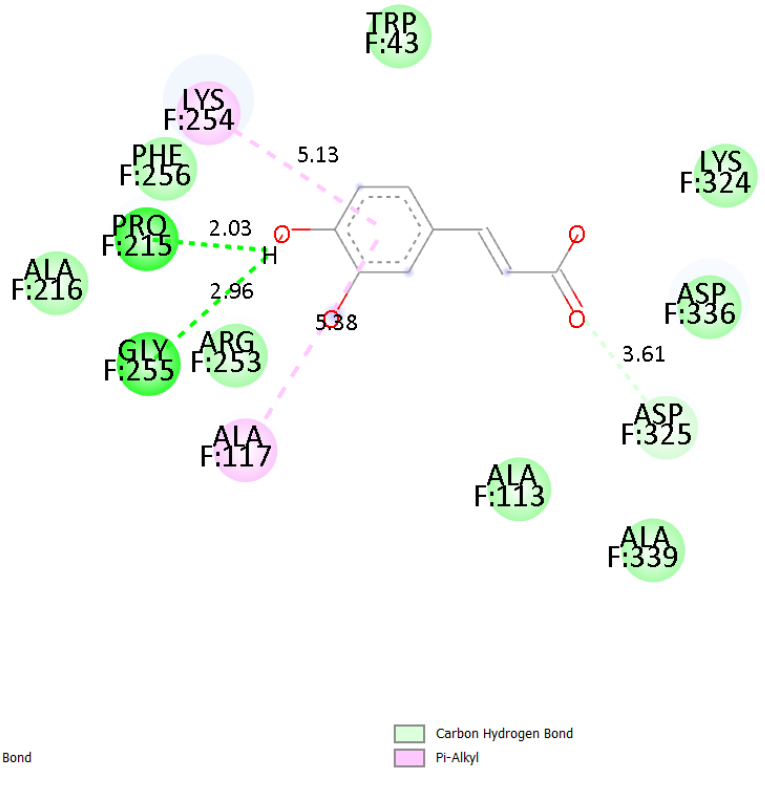


FIGURE 4.14: Molecular Docking analysis of Fusion protein and Caffeic acid (a) 3D structure of hMPV Fusion protein (b) 3D structure of Caffeic acid (c) SAS view of hMPV Protein and Caffeic acid complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

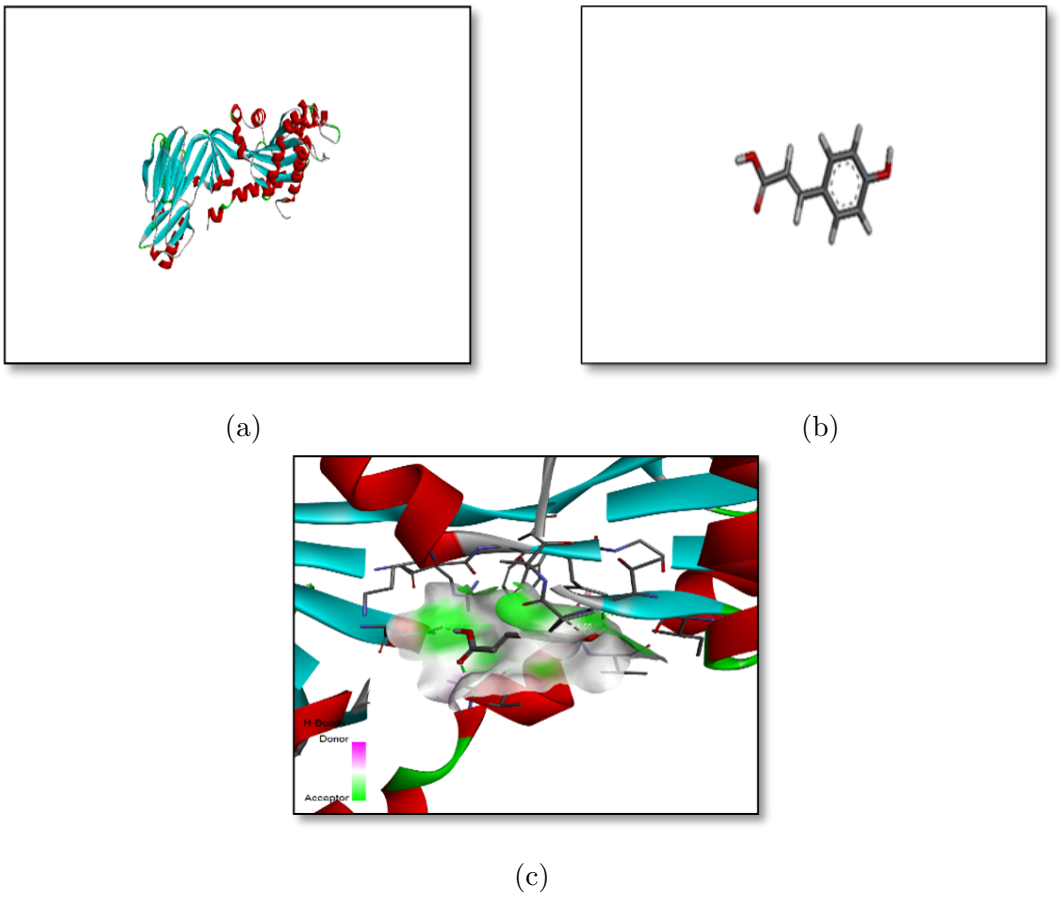


FIGURE 4.15: Molecular Docking analysis of Fusion protein and p- coumaric acid (a) 3D structure of hMPV Fusion protein (b) 3D structure of p-coumaric acid (c) SAS view of hMPV Protein and p- coumaric acid complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

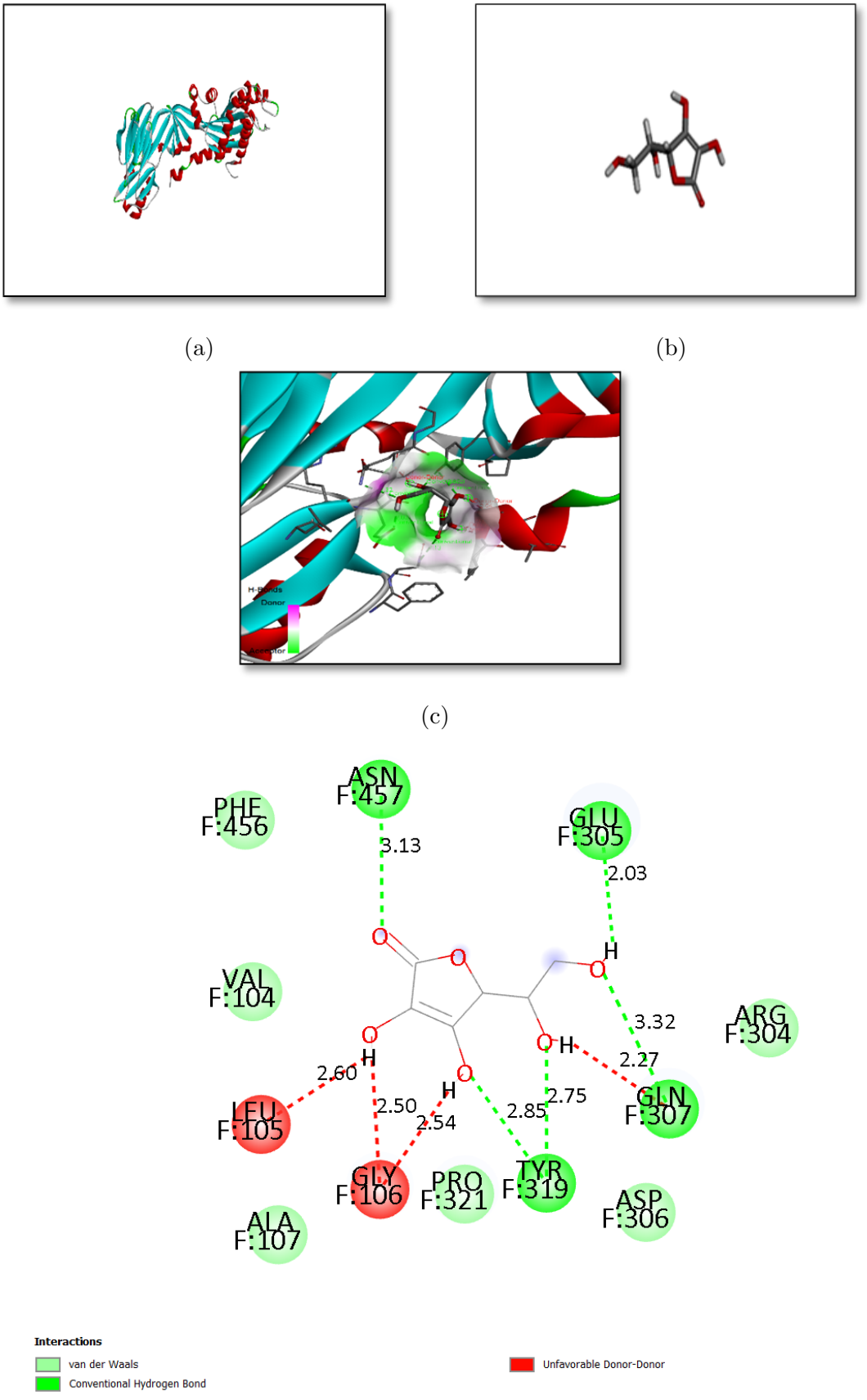


FIGURE 4.16: Molecular Docking analysis of Fusion protein and L- ascorbic acid (a) 3D structure of hMPV Fusion protein (b) 3D structure of L-ascorbic acid(c) SAS view of hMPV Protein and L-ascorbic acid complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

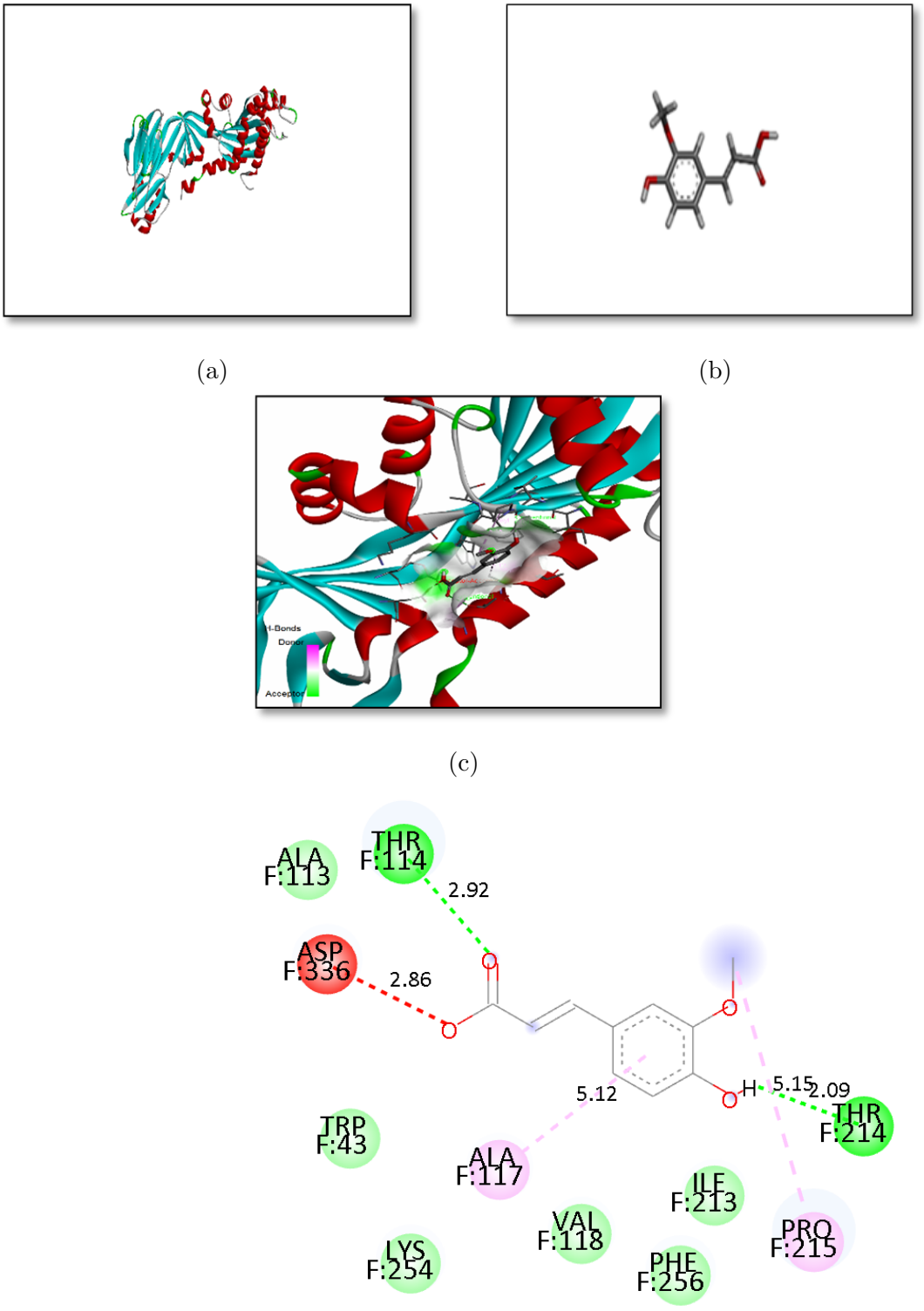


FIGURE 4.17: Molecular Docking analysis of Fusion protein and Ferulic acid (a) 3D structure of hMPV Fusion protein (b) 3D structure of Ferulic acid (c) SAS view of hMPV Protein and Ferulic acid complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

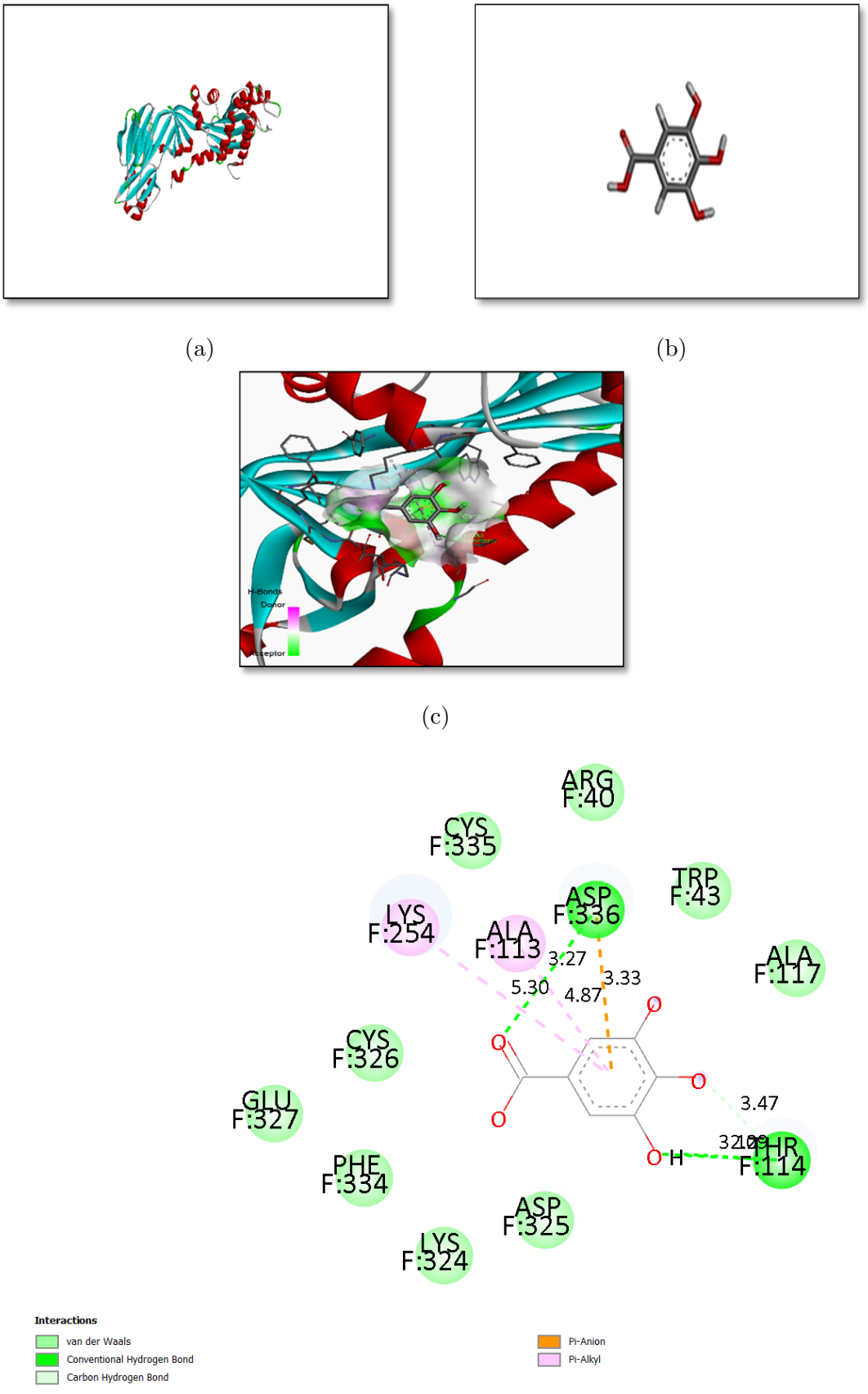


FIGURE 4.18: Molecular Docking analysis of Fusion protein and Gallic acid (a) 3D structure of hMPV Fusion protein (b) 3D structure of Gallic acid (c) SAS view of hMPV Protein and Gallic acid complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

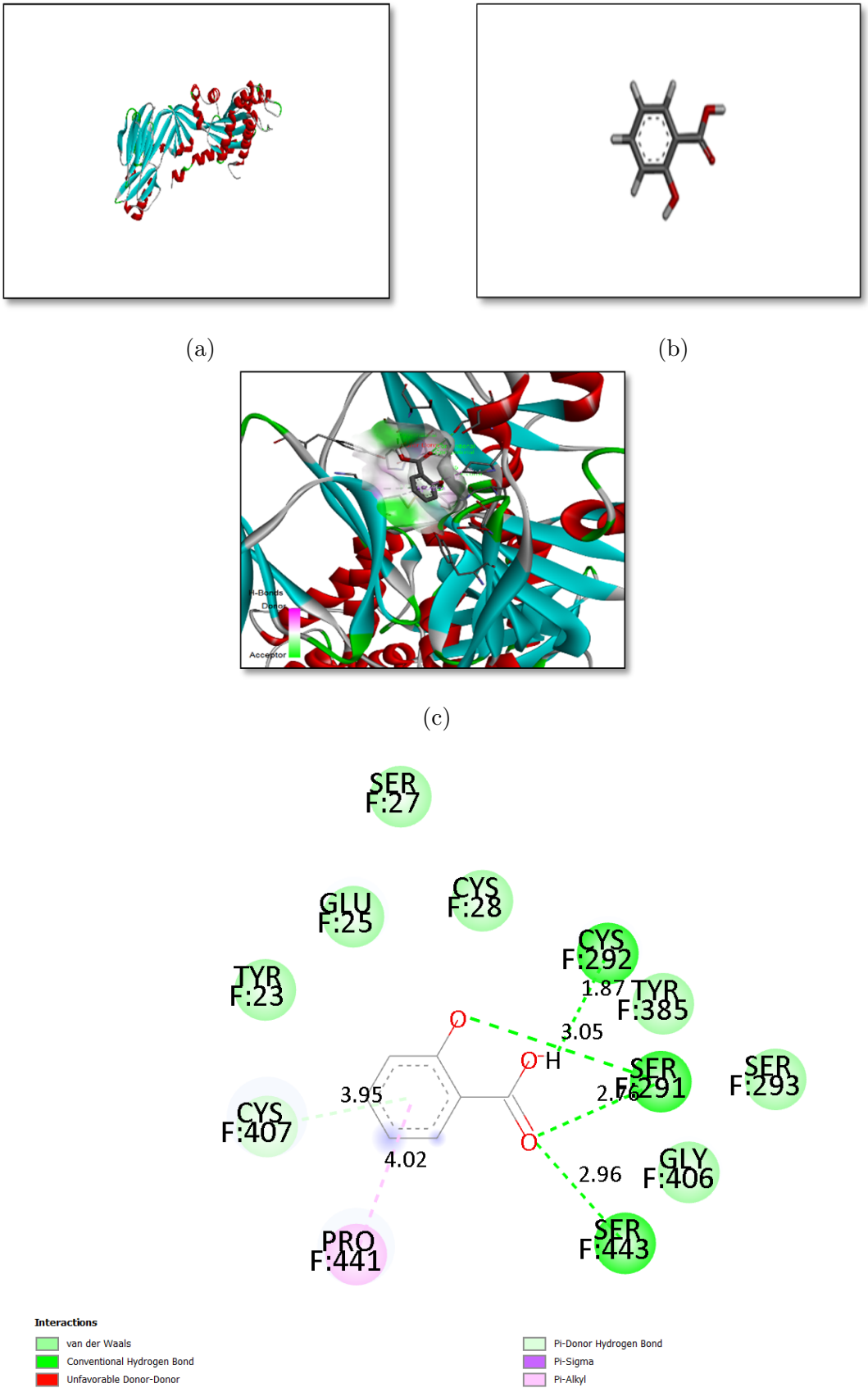
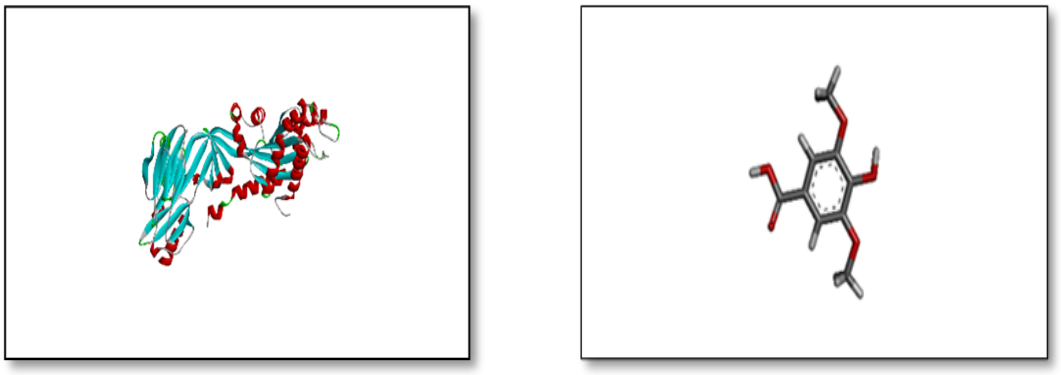


FIGURE 4.19: Molecular Docking analysis of Fusion protein and Salicylic acid (a) 3D structure of hMPV Fusion protein (b) 3D structure of Salicylic acid (c) SAS view of hMPV Protein and Salicylic acid complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.



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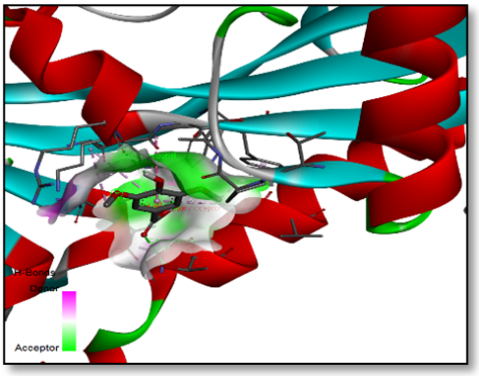

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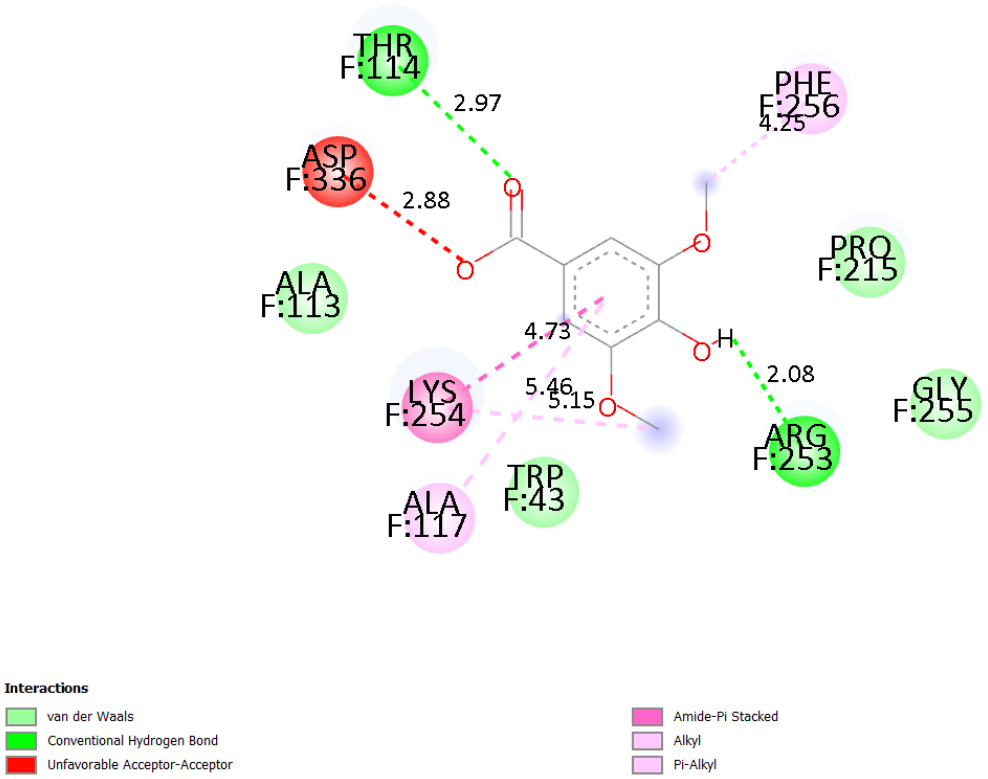


FIGURE 4.20: Molecular Docking analysis of Fusion protein and Salicylic acid (a) 3D structure of hMPV Fusion protein (b) 3D structure of Salicylic acid (c) SAS view of hMPV Protein and Salicylic acid complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

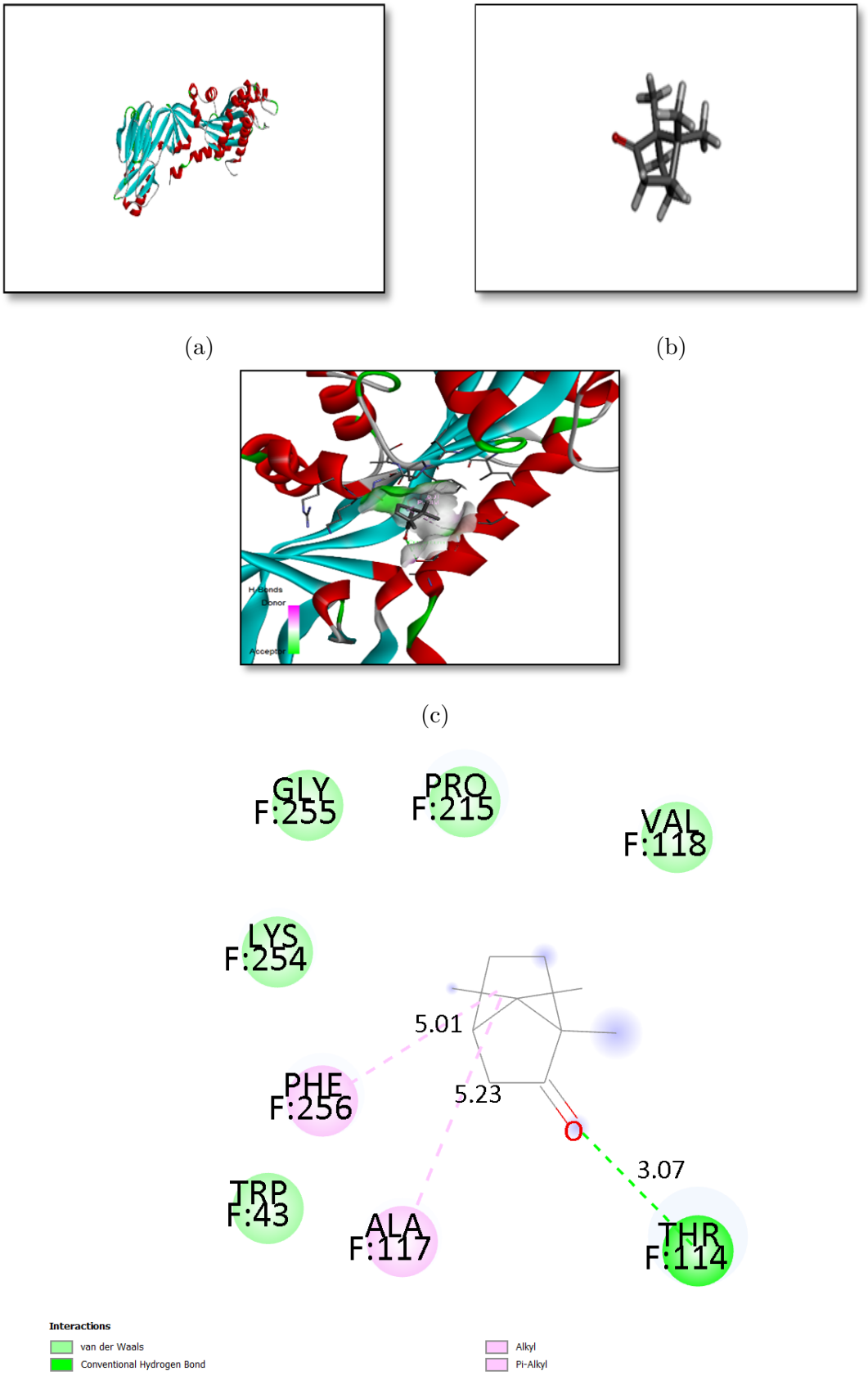


FIGURE 4.21: Molecular Docking analysis of Fusion protein and Camphor (a) 3D structure of hMPV Fusion protein (b) 3D structure of Camphor (c) SAS view of hMPV Protein and Camphor complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

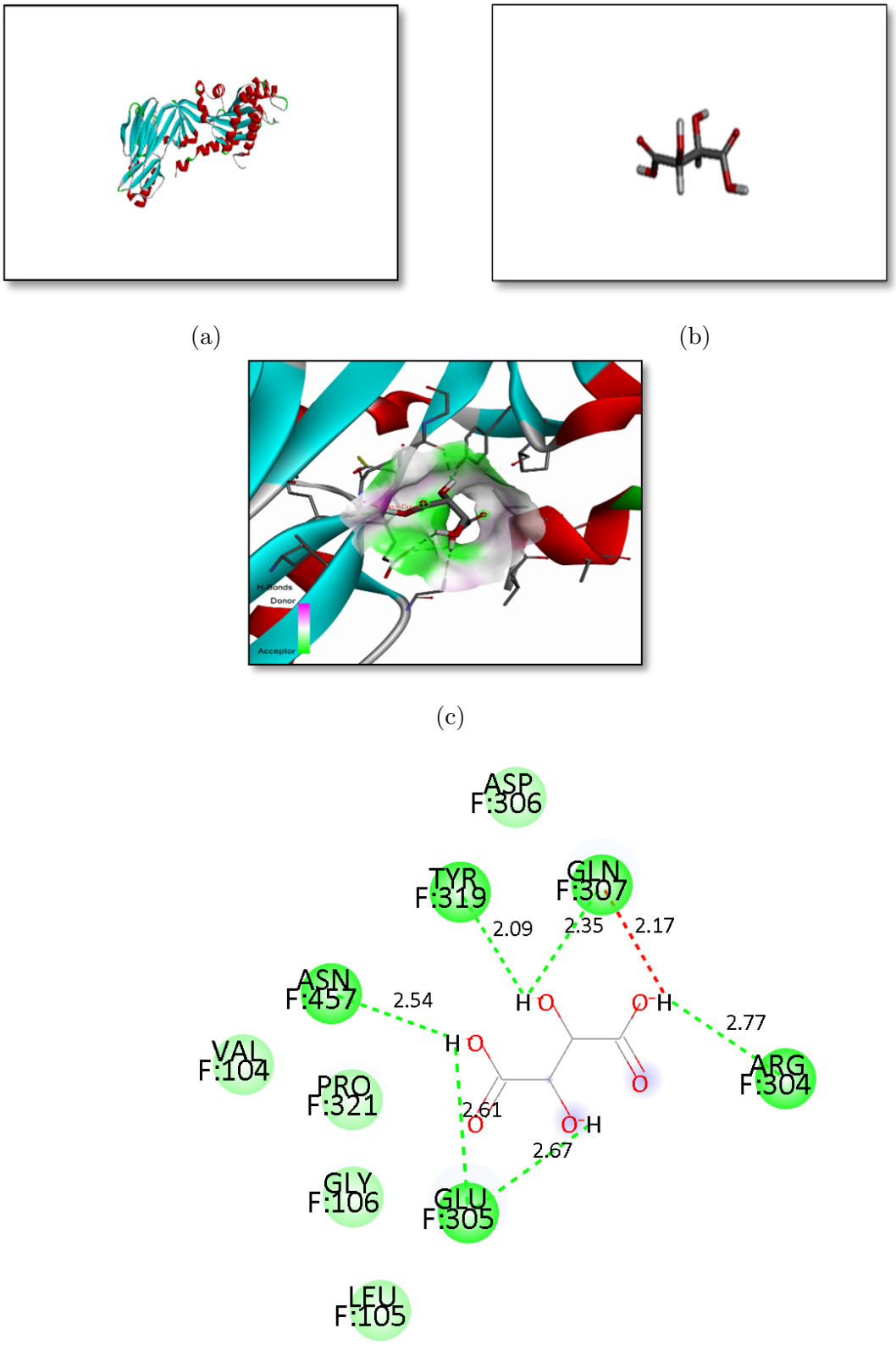
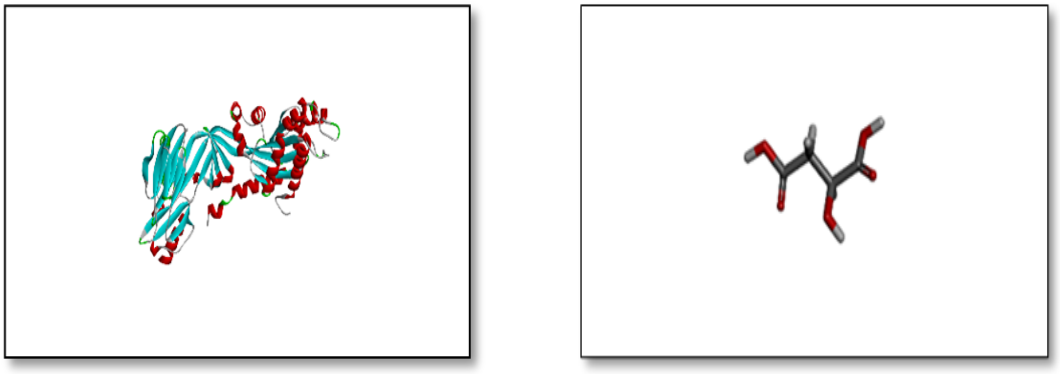
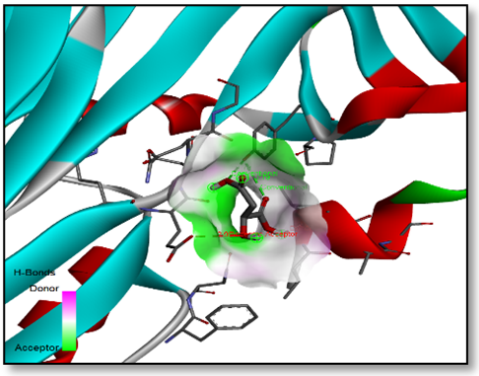


FIGURE 4.22: Molecular Docking analysis of Fusion protein and Tartaric acid (a) 3D structure of hMPV Fusion protein (b) 3D structure of Tartaric acid(c) SAS view of hMPV Protein and Tartaric acid complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.



(a) (b)



(c)

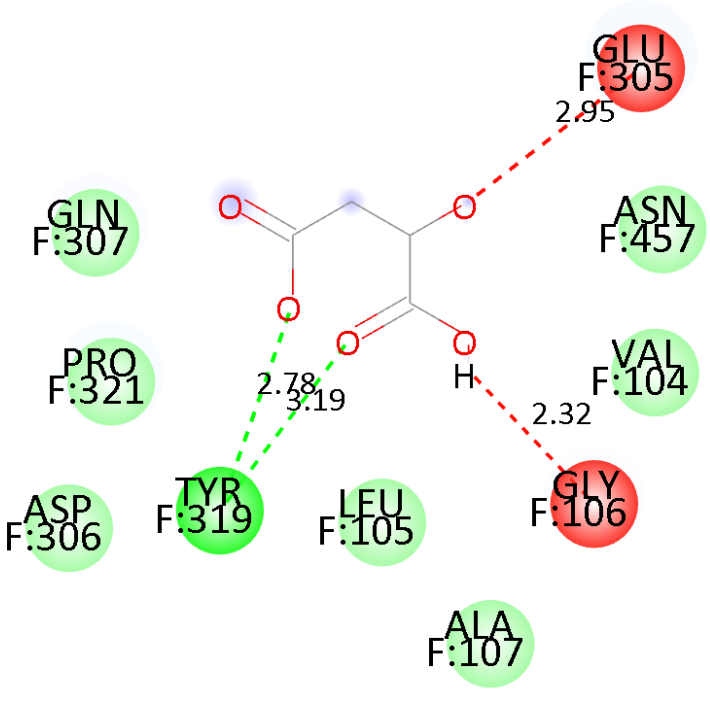
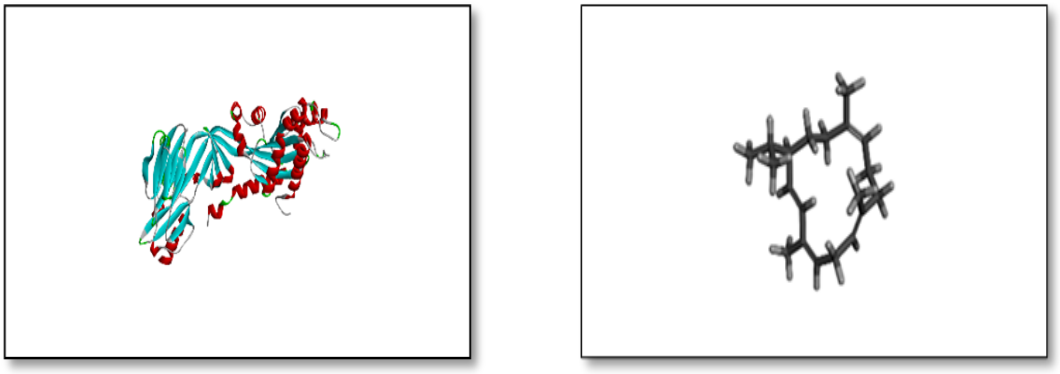
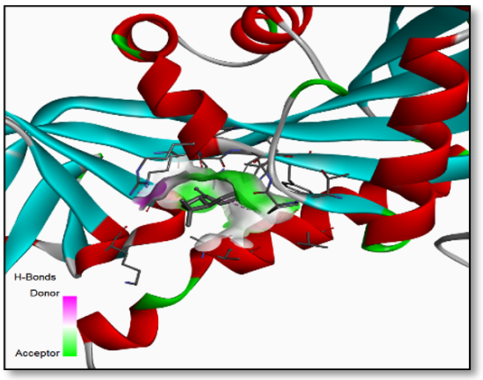


FIGURE 4.23: Molecular Docking analysis of Fusion protein and Mallic acid (a) 3D structure of hMPV Fusion protein (b) 3D structure of Mallic acid (c) SAS view of hMPV Protein and Mallic acid complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.



(a) (b)



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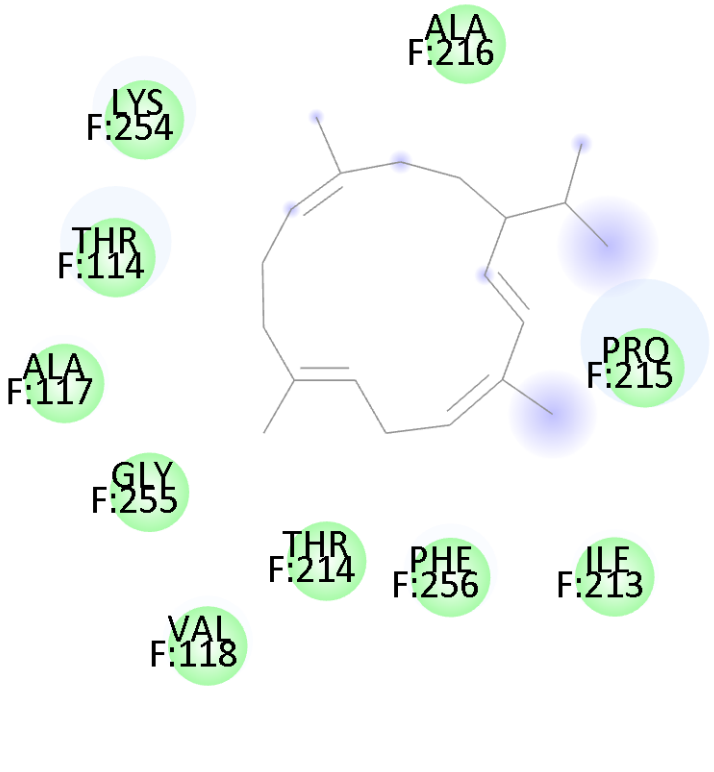
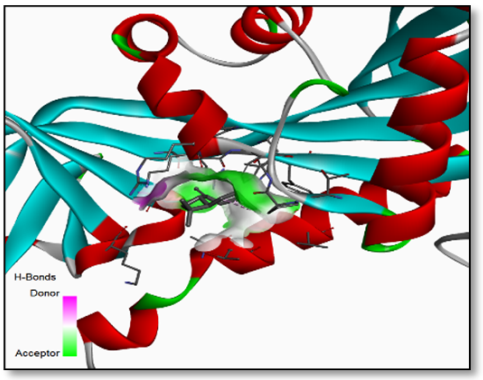


FIGURE 4.24: Molecular Docking analysis of Fusion protein and Cembrene (a) 3D structure of hMPV Fusion protein (b) 3D structure of Cembrene (c) SAS view of hMPV Protein and Cembrene complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

(a) (b)



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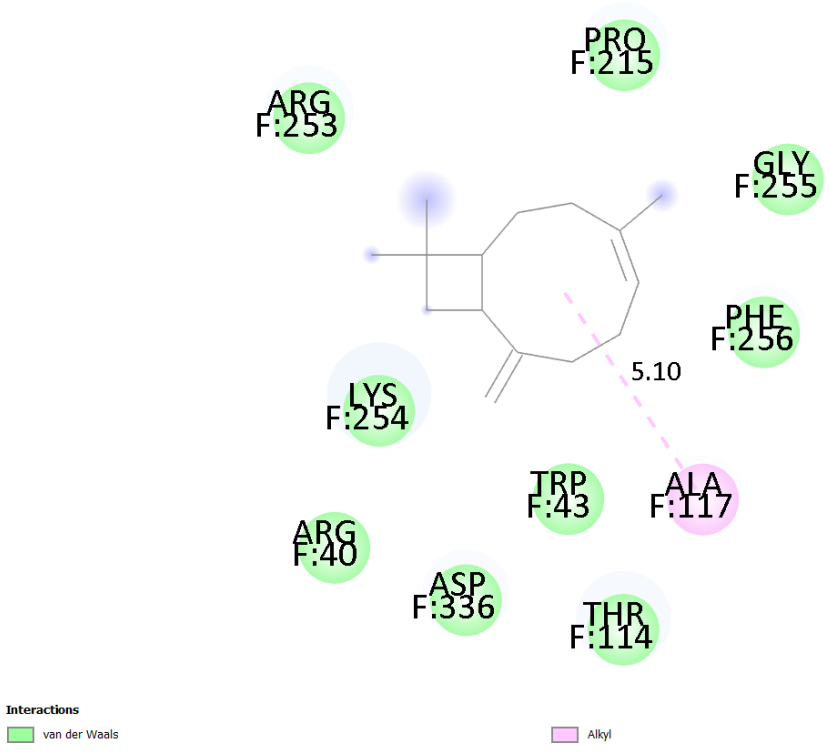

FIGURE 4.25: Molecular Docking analysis of Fusion protein and E-caryophyllene (a) 3D structure of hMPV Fusion protein (b) 3D structure of E-caryophyllene (c) SAS view of hMPV Protein and E-caryophyllene complex (d) 2D interaction of active site of Ligand and amino acids residues of Fusion protein.

4.4 Lead Compound Identification

Virtual screening was done by using Lipinski's rule of 5 and ADMET properties were also predicted. As secondary filter, binding scores were used to identify lead compound. Among all of the selected 30 ligands Sumaflavone, Amentoflavone, Narirutin, Rutin, Cyanidin - 3 - rutinoside, Quercetin - 3 - O - glucose, Delphinidin - 3 - glucoside were not obeying the Lipinski rule so they were dropped.

Cyanidin-3-glucoside has shown the greatest binding energy -7.9 but it was having very poor absorption so it was knocked out. After analyzing the ADMET properties and binding energies both Quercetin (-7.7) and Myricetin (-7.8) were selected as Lead compounds because they owned strong binding energies along with good absorption and safety profile. Later simulation was performed to understand the detailed interaction between protein and lead compounds.

4.5 Results of Molecular Dynamic Simulations

4.5.1 Molecular Dynamics Analysis of Quercetin-Protein Complex Stability

The simulation of 5WB0 protein with Quercetin was run under physiological conditions (NPT ensemble, 310 K, 1 atm) for 200 nanoseconds. The system was solvated in a water box with ∼20,093 water molecules and neutralized using Na^+ and Cl^- ions at a near-physiological salt concentration (∼51 mM). Quercetin ($C_{15}H_{10}O_7$) is a natural flavonoid, with several rotatable bonds and hydroxyl (-OH) groups that are crucial for binding.

A 200-nanosecond molecular dynamics (MD) simulation was carried to measure the stability and binding characteristics of Quercetin in the target protein's active site. The study indicated the creation of a stable protein-ligand combination, characterized by minimal structural drift and a persistent network of molecular interactions.

After the first 80 ns, the simulation reached a stable equilibrium state, as shown by the (RMSD) Root Mean Square Deviation of the protein backbone, a measure of global structural drift, equilibrating at roughly 1.5-2.0 Å(Figure 4.26).

Simultaneously, the ligand RMSD, which was computed following protein backbone alignment, continuously stayed below 2.0 Å. This low positional deviation confirms that quercetin remained tightly bound within its original binding pocket without significant dissociation or translational movement.

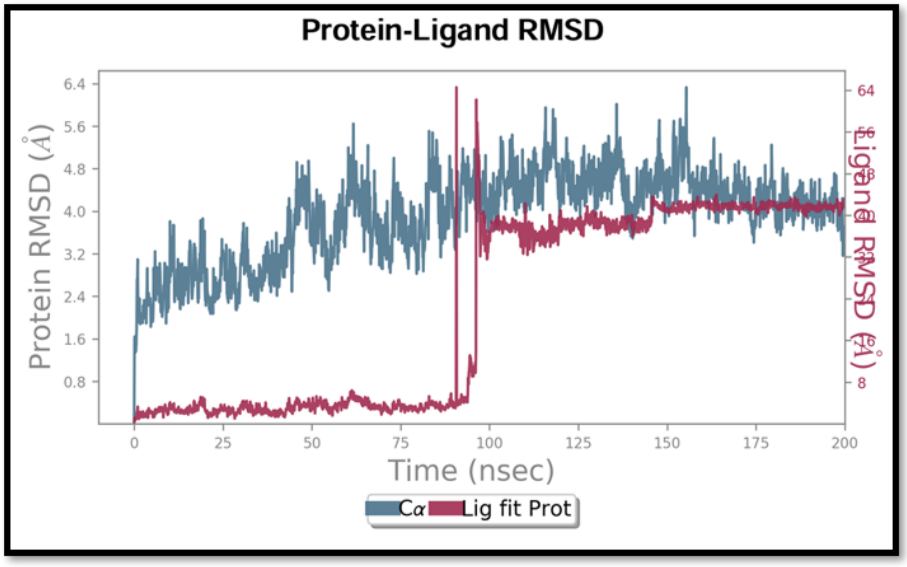

FIGURE 4.26: Analysis of Protein Ligand complex RMSD (Quercetin and 5WB0)

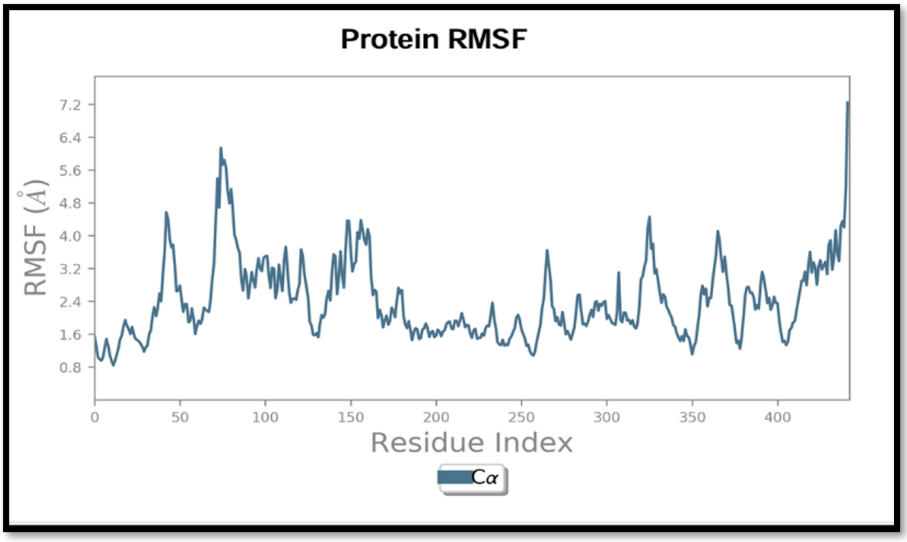

FIGURE 4.27: Root Mean Square Fluctuation (RMSF) of 5WB0

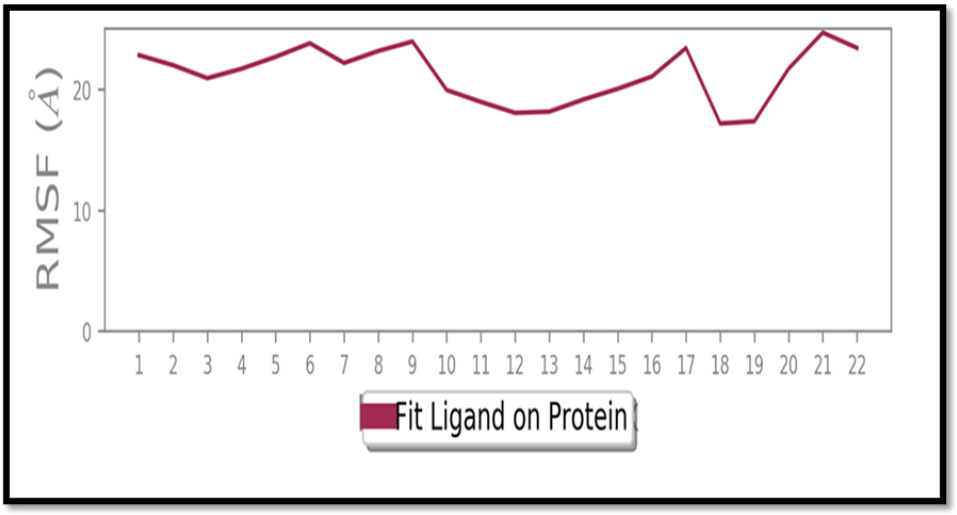

FIGURE 4.28: RMSF of Quercetin on 5WB0

The ligand Root Mean Square Fluctuation (RMSF) profile presented a clear pattern atoms in the aromatic core showed very little flexibility (< 0.5 Å), indicating that this scaffold was firmly attached. Peripheral hydroxyl groups, on the other hand, exhibited considerable changes (1.0-1.8 Å), which is consistent with their function in creating dynamic, adaptable hydrogen bonds (Figure 4.28). The residues that make up the binding site (such as those in areas 290-300 and 325-332) showed low to moderate variations (1.0-2.0 Å), according to complementary study of protein RMSF, indicating that the pocket remained structurally well-defined. Higher flexibility was seen in the loop and terminal areas, which is consistent with intrinsic protein dynamics. Throughout the simulation, the makeup of the protein's secondary structure remained remarkably consistent. There was no discernible unfolding or loss of structural features over time, and the average percentage of alpha helices and beta strands remained steady at 44.6%. (Figure 4.27).

Important residues that stabilize the complex were found by a thorough examination of protein-ligand interactions. ASP331, GLU294, LYS295, TYR299, ARG329, HIS332, and TYR385 were the most stable connections that persisted throughout the simulation. Hydrogen bonds accounted for the majority of contacts, according to interaction type analysis, with hydrophobic interactions coming in second. This profile is exactly in line with the chemical structure of quercetin, which is abundant in aromatic rings and hydroxyl groups that form hydrogen bonds. For more over

half of the simulation duration, quercetin's hydroxyl and central carbonyl groups interacted with ASP331, whereas other hydroxyl groups interacted with GLU294, TYR299, ARG329, and HIS332. Water-bridged interactions were present but not dominant, suggesting quercetin effectively displaces solvent molecules from the binding pocket, a favorable characteristic for high-affinity binding. (Figure 4.29)

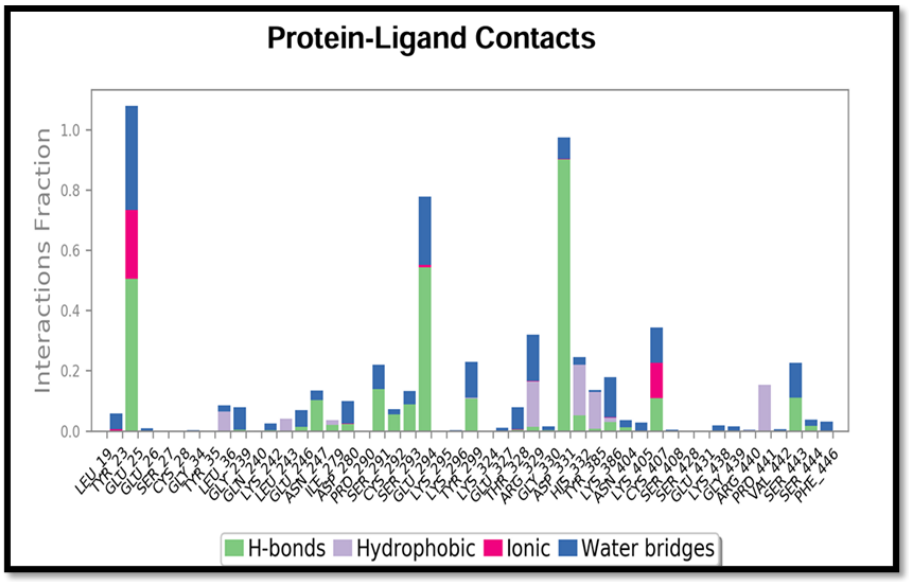

FIGURE 4.29: 5WB0 and Quercetin Contact

Several measures were used to evaluate the bound ligand's solvent exposure and compactness. Quercetin maintained a constant, folded shape without unfolding, as evidenced by the radius of gyration remain steady at roughly 3.8 Å(Figure 4.30). Polar surface area (PSA) and solvent-accessible surface area (SASA) stabilized at around 110 $Å^2$ and 210 $Å^2$, respectively. The relatively low and stable PSA value indicates that the ligand's polar groups are predominantly engaged in protein interactions rather than exposed to solvent, further confirming its deeply buried position within a hydrophobic binding pocket.

These findings show that quercetin and the target protein create a stable and well-defined complex. Minimal overall structural drift, a firmly retained ligand core, a clearly defined binding site, and a robust network of particular hydrogen bonds and hydrophobic interactions with important amino acid residues all contribute to the stability.

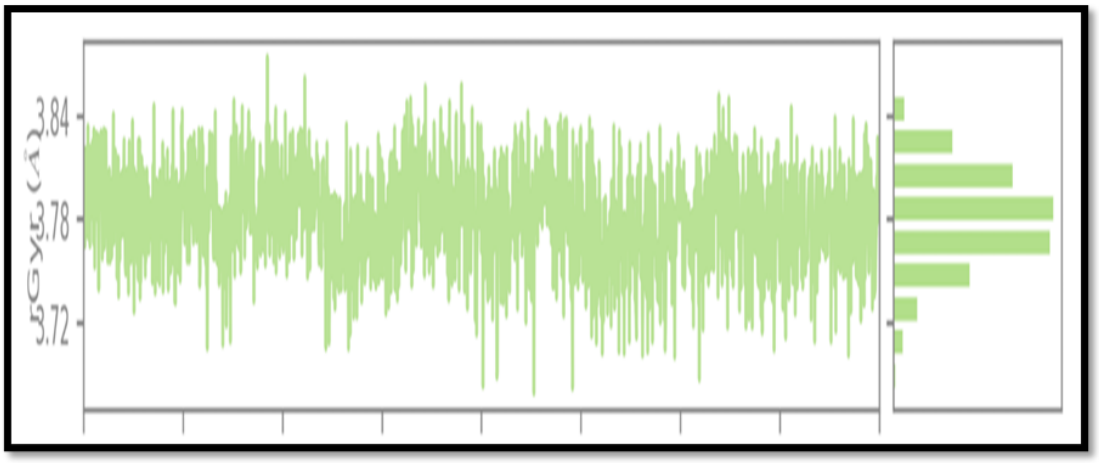

FIGURE 4.30: Radius of Gyration of Docked Complexes (Quercetin and 5WB0)

4.5.2 Molecular Dynamics Analysis of Myricetin - Protein Complex Stability

RMSD of protein remain stable within an acceptable range (1–3 Å) after equilibration. Structure of protein is conformational unchanged and stable with no denaturation and unfolding. Residues at binding site has shown relatively lower flexibility while higher fluctuations are seen in terminal and loop regions. Well defined binding pockets were observed suitable for stable binding of ligand. The total secondary structure comprises 42.84% (with 19.05% helix and 23.79% strand), showing stability throughout the simulation and indicating no significant loss of secondary structure, thus confirming the overall firmness of the protein fold. (Figure 4.31)

The RMSD of ligand for heavy atom RMSD remains low ($\leq$ 3 Å) comparative to the protein. Myricetin keeps a stable pose within the binding pocket without important displacement. The RMSF of the ligand indicates that fluctuations differ for each atom, with polar hydroxyl groups exhibiting greater flexibility than the rigid aromatic scaffold. The ligand shows dynamic adaptation while preserving core interactions, indicating potential entropic contributions to binding. (Figure 4.32 and Figure 4.33).

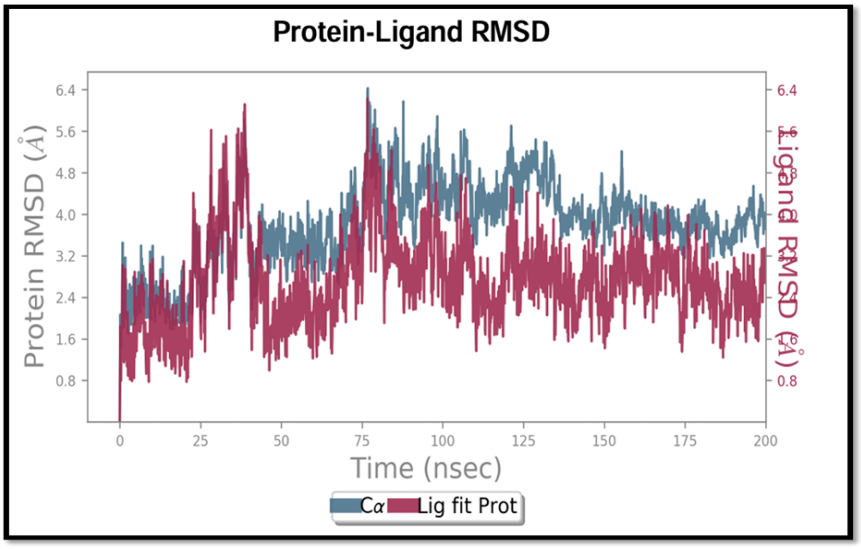

FIGURE 4.31: Analysis of Protein Ligand complex RMSD (Myricetin and 5WB0)

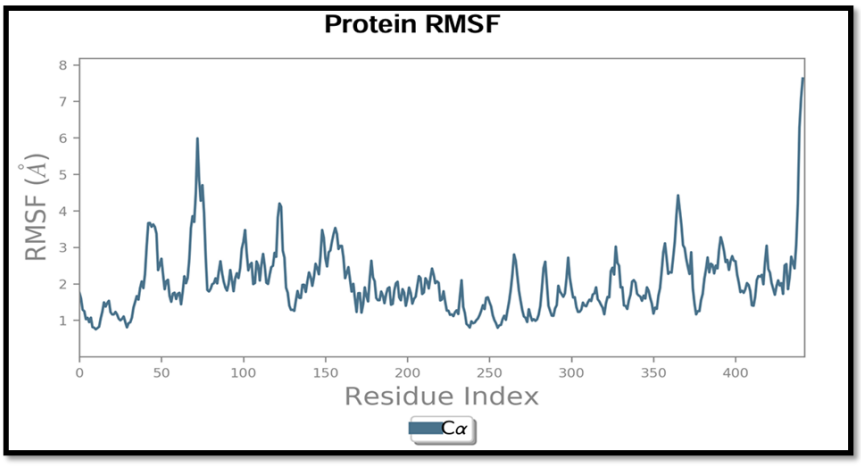

FIGURE 4.32: Analysis of Root Mean Square Fluctuation (RMSF) of 5WB0

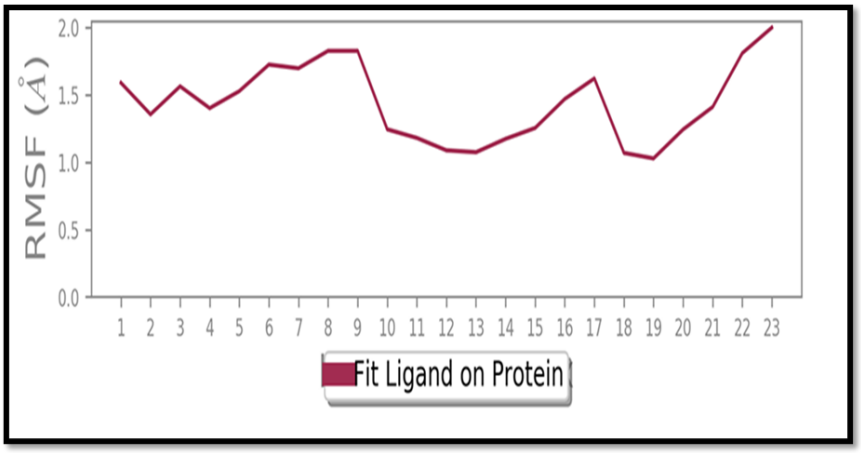

FIGURE 4.33: RMSF of Myricetin on 5WB0

The main residues involved were TYR_23, GLU_25, SER_291, SER_293, GLU_294, LYS_295, TYR_299, TYR_385, LYS_386, GLY_387, ASN_404, LYS_405, CYS_407, SER_408, LYS_438, GLY_439, ARG_440, PRO_441, SER_443, SER_444, PHE_446. Multiple serine, tyrosine, and glutamate residues were involved in the predominant interaction type with persistent H-bonds. Aliphatic interactions and aromatic residues (TYR, PHE) contributed significantly to the hydrophobic connections. Partial solvent displacement was indicated by the presence but lack of predominance of water bridges. Ionic interactions are minimal and consistent with the neutral charge of the ligand. Many interactions are sustained for more than 30% of the simulation time; some residues, like ARG_440, exhibit >100% because of several contacts occurring at once.

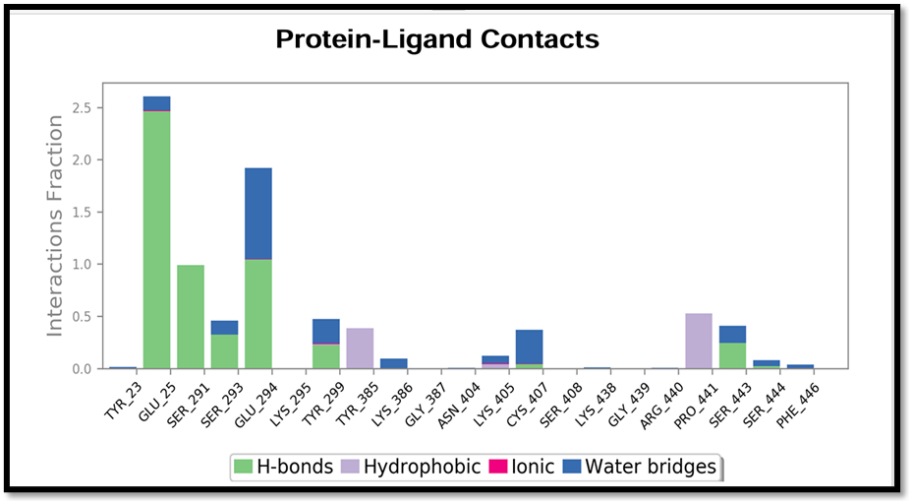

FIGURE 4.34: 5WB0 and Myricetin Contact

These findings show that during 200 ns of simulation, myricetin forms a stable complex with Chain F of 5WB0. Important stabilizing interactions include hydrophobic contacts with aromatic residues (TYR, PHE) and hydrogen bonds with polar residues (SER, TYR, GLU, ARG) (Figure 4.34).

The ligand conformational properties of myricetin shows that a compact bound conformation is present which is seen by the presence of stable radius of gyration (∼4.2Å) throughout the simulation. The ligand was partially buried within a hydrophobic binding pocket, with its polar groups involved in protein interactions rather than solvent exposure, according to the solvent-accessible surface area

(SASA ∼180[12]) and polar surface area (PSA ∼95Å^2) values. Furthermore, the ligand's internal conformational stability while attached to the protein was probably aided by the development of intramolecular hydrogen bonds between its hydroxyl groups (Figure 4.35).

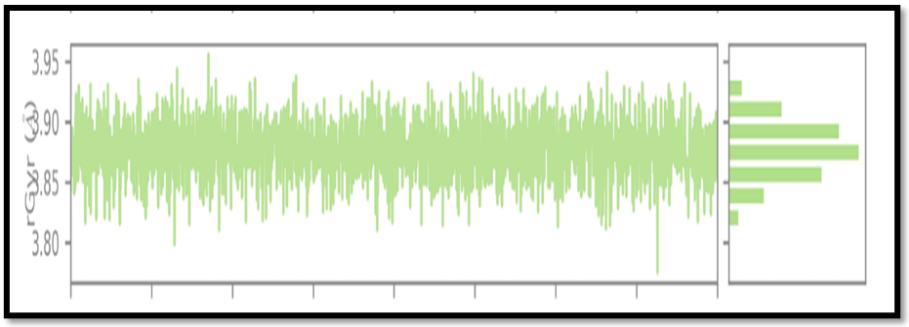

FIGURE 4.35: Radius of Gyration of Docked Complexes (Myricetin and 5WB0)

4.5.3 MD Simulation Comparison Between Myricetin and Quercetin

Comparison of myricetin and quercetin shows that the stable complexes are formed by both ligands. Quercetin shows slightly low RMSD suggesting a tighter fit with good binding stability. Myricetin engages a wide set of residues including N-terminal interaction because of its additional OH group representing diversify in interaction. Additionally, Myricetin is more deeply buried with lower polar surface area indicating more hydrophobic complementarity. Myricetin's peripheral hydroxyls are more flexible, which could result in an entropic penalty but also enable adaptive binding. Both myricetin and quercetin exhibit durable interaction networks, well-maintained protein structure, and stable binding to the target protein.

Chapter 5

Discussion

Human Metapneumovirus (hMPV) is a significant respiratory pathogen, particularly affecting children, the aged and immunocompromised individuals. The lack of approved vaccinations or targeted antiviral therapies for hMPV, considering its clinical significance, demands the investigation of novel approaches for treatment.

Flavonoids are known as famous therapeutic and prophylactic agent against wide range of old and new viruses. About 10,000 natural flavonoids have been recognized, being plant-based phytoconstituents. They have fewer side effects as compared to traditional anti-viral agents which are more effective against viral diseases [224].

Quercetin did not violate any of the Lipinski's rule of 5 which is the indication of positive drug likeness of the compound. While on the other hand, myricetin only violated one Lipinski's rule i.e. less than 5 hydrogen bonds but this is still possessing positive drug likeness [225]. Due to the structural compatibility with CYP1A2's active site, which supports planar and polycyclic compounds with electron-dense aromatic groups, quercetin and myricetin are biologically specific for this enzyme [226]. Meanwhile their planar shape and general hydrophobicity facilitate stable binding within the catalytic region of the enzyme. The aforementioned flavonoids both get metabolized by CYP1A2 and act as its moderate

inhibitors, competitively interfering with the metabolism of clinical medications such as theophylline and clozapine [227]. This interaction is functionally dualistic.

Quercetin and myricetin have high hydrophilicity and poor membrane division, which are caused by multiple ionizable phenolic hydroxyl groups (in particular, the catechol moiety on the B-ring), are the main causes of their low CaCo2 permeability. At physiological intestinal pH, these molecules are primarily in a polar, ionized state, which severely restricts passive transcellular diffusion [228, 229]. The combination of its strong polarity, numerous hydroxyl groups, active efflux via P-gp/MRP2, and substantial intestinal metabolism, quercetin and myricetin have low Caco-2 permeability, which inhibits passive diffusion and oral bioavailability. In-silico techniques including P-gp substrate prediction, prodrug design, and selective hydroxyl masking may increase permeability to maximize these leads. While maintaining function, formulation methods like nanoparticles could enhance intestinal absorption even further.

Myricetin and quercetin's well-established interaction with this crucial efflux transporter can be seen in the P-glycoprotein (P-gp/ABCB1) substrate classification of "Yes" for both compounds. This is mainly because their distinct physicochemical and structural characteristics align with P-gp's substrate recognition profile. P-gp prefers moderately lipophilic, amphipathic substrates that can intercalate into the transporter's transmembrane domains, and flavonoids such as quercetin and myricetin possess a planar, polycyclic scaffold with amphipathic character that includes both hydrophobic aromatic rings and multiple hydrophilic hydroxyl groups [230, 231]. Therefore, the positive response is biologically significant for flavonoid disposition, therapeutic efficacy, and drug interaction risk, theoretically supported by structural compatibility, and scientifically validated by transport and inhibitory research studies.

Quercetin is a natural compound possessing antioxidant, anti-inflammatory and antiviral properties have revealed promising effects against hMPV. Recent studies have suggested that quercetin may have the ability to develop new mitigating approaches for managing severity of hMPV infections by targeting inflammation, oxidative stress, and replication of virus. All mentioned mechanisms can worsen

lung damage and provide a suitable environment for hMPV replication. While quercetin confirmed the protective effects against oxidative damage in many diseases. It has shown to inhibit the viral replication and helps in modulating immune response to infections caused by influenza, RSV and rhinovirus [232].

Due to high bioavailability and solubility quercetin is increasingly used in many novel preparations for human health care [233]. Moreover, because of its prominent antiviral properties in reducing DNA gyrase, inhibiting polymerases, reverse transcriptase, proteases, and binding viral capsid, quercetin has been investigated in a variety of viral infection types and models [234].

A wide range of tiny molecules which are obtain from plants are recognized for their antiviral properties [235] [236]. Although no proper antiviral drugs extracted from plants' constituents have been approved. Main reason for this could be problem faced in safety and efficiency of herbal medications because of herb-drug interaction and herbal adverse events [237].

Quercetin has recently been employed as an accessary medication for COVID-19 symptomatic individuals; this has improved clinical symptoms and shortened hospital stays [235].

African swine fever virus (ASFV) protease was inhibited by myricetin with an IC50 value of 8.4μM [238]. Herpes simplex virus HSV-2 has highly immunogenic glycoprotein d (gd) which plays important role in viral entry into host cells. Recent studies have shown the ability to directly interact with viral gd protein which results in blocking adsorption of virus and fusion of membrane to host cell. Additionally, myricetin inhibits viral infection and replication by down-regulating the host (epidermal growth factor receptor/phosphoinositide 3-kinase/Akt or protein kinase B) signaling path [239].

Research has shown the positive result extract from elderberry on influenza virus H1N1 in MDCK (Madin–Darby canine kidney) cells. The main constituent in myricetin was (±) dihydromyricetin which inhibited the virus with an (IC50 value) 8.7μM [240]. Replication of Zika virus was inhibited by myricetin after inhibition of viral RNA production [241]. Enterovirus A71 (EV71) infectivity is said to

be modulated by myricetin and dihydromyricetin, however the exact method of inhibition is unknown [242].

Myricetin rhamnoside (MyrG) and myricetin-3α-O-rhamnosyl (1→6)-α-galactoside (MyrGG), which are derived from Marcetia taxifolia DC. (Melastomataceae), have been reported to suppress the Hepatitis B virus (HBV) [243]. Myricetin-3-O-rhamnoside, which was extracted from the leaves of *Guiera senegalensis* (Combretaceae), has also been shown to have in vitro anti-HBV action [244]. Myricetin from *Dioscorea bulbifera L.* (Dioscoreaceae) and *Marcetia taxifolia* have been shown to have strong inhibitory properties against HIV-1 integrase and reverse transcriptase (RT) [245]. Myricetin's glycosylated component might encourage cell internalization and boost anti-HIV-1 action.

Another research reported the analysis of interaction between nonstructural protein NS1 and quercetin. The RSV non-structural protein NS1 is essential in regulating the host's reaction to disease and opposing the interferon-mediated antiviral state [246]. The interaction among RSV-NS1 and quercetin is long-lasting, with a dissociation constant on the order of 10-6 M, based on numerous insilico and experimental approaches. Therefore, NS1 may be a possible target for quercetin [247]. Based on a study, quercetin and its derivatives might interact with the M2-1 protein, which plays a role in transcription and genome replication through the creation of complex RNA-dependent RNA polymerase. Molecular docking results indicate that these substances can influence M2-1's activity in key areas [248] [249]. Quercetin and acetylated derivatives are considered as an alternative anti-RSV strategy found which might interact with F protein on Human respiratory syncytial virus (RSV) surface in crucial region for viral infection and attachment. Acetylated quercetin interacted with F-protein through molecular docking, showing ΔG = -14.22 kcal/mol and being more stable than quercetin [250].

Using an in vitro technique, a possible therapeutic function of dietary antioxidants in hMPV infection was studied. Pro-inflammatory cytokines such IL-1, IL-6, and TNF-α, as well as the chemokines CXCL10 (IP-10) and CCL4 (MIP-1), have been reported to be significantly higher in hMPV infection. Cytokines and chemokine excretion from infected cells are significantly reduced by quercetin

treatment. Furthermore, compared to untreated infected cells, quercetin administration was linked to a significantly decreased viral titer. According to the findings of this research, an antioxidant diet can reduce oxidative damage and inflammatory reactions also interfering in viral assembly and mature virus generation [251]. To sum up, quercetin may have an antiviral effect via interacting with both non-structural and structural proteins also decreasing pro-inflammatory cytokines.

An invitro study was conducted by Peng et al to assess the antiviral activity of 4 flavonoids myricetin, luteolin, epigallocatechin gallate and rutin against Ranavirus (RGRV). All of them have shown antiviral activity but myricetin has demonstrated the most powerful antiviral activity. Moreover, myricetin has demonstrated the inhibition of RGRV infection at all stages particularly at early viral replication by down regulation and delaying of early genes expression [252].

It is generally known that MD simulations can be utilized for confirming docking results. MD stability has become an essential prerequisite for differentiating between transitory and functionally meaningful ligand binding in similar computational studies that target hMPV or related respiratory virus proteins. For instance, comparative MD analyses validated quercetin's potential as a lead antiviral candidate by showing that it maintained stable interactions with the hMPV Fusion protein with low RMSD and sustained hydrogen bonding patterns [217].

Chapter 6

Conclusion and Future Recommendations

6.1 Conclusion

Human metapneumovirus (hMPV) is the most chief reason of respiratory tract infection predominant in infants, elderly and individuals who have weak immune system. Human metapneumovirus hMPV known as broad spectrum respiratory infection which causes severe lower and mild upper respiratory infections including pneumonia and bronchiolitis. This causes infection in senior population and immune compromised individuals. Despite of global impact of hMPV as global hazard there are no approved vaccines or antiviral drugs to combat the harmful effects of hMPV. So, this is the need of time for developing therapeutic strategies.

To achieve first objective of the study was identification and retrieval of bioactive compounds of *R. coriaria* from PubChem. For these 30 compounds with reported medicinal properties were retrieved from respective database. The second objective was to identify and prepare 3D structure of hMPV's Fusion protein (F0) from PDB which was attained by retrieval of protein and saving it in pdb format. Third objective for this study was to perform virtual screening by Lipinski's rule of 5 and ADMET properties analysis and performing molecular docking. In this

study, 30 bioactive compounds derived from *R. coriaria* were screened initially for their potential antiviral activity against hMPV by using Lipinski's rule of 5. Later ADMET profiling was done for rest of 23 compounds for confirming the absorption, distribution, metabolism and excretion and toxicity characteristics for further selection of compounds.

Following the preliminary screening molecular docking was done by PyRx as secondary evaluation by finding out the binding affinities and interactions between the amino acid of target protein and ligands suggesting their anti-viral activity. Among all compounds Myricetin and Quercetin have shown optimal drug likeness and favorable pharmacokinetic properties along with strong binding affinities of (-7.8 and -7.7 respectively) with target viral fusion protein, so these compounds were selected as Lead compound of study.

Fourth objective was to run MD simulation for identified Lead compounds to evaluate the structure stability of protein ligand complex. For further validation of these finding under dynamic physiological conditions molecular dynamic MD simulations were performed for Lead compounds (Myricetin and Quercetin). The MD simulation results verified the structural stability of protein ligand complex over the period of 200ns with sustained interactions and minimal conformational deviation.

The finding has shown strong binding stability and favorable conformational behavior of Myricetin and Quercetin with Fusion protein with acceptable root mean square deviation (RMSD) with, root mean square fluctuation (RMSF) and consistent intermolecular interaction throughout the simulation period. ASP331, GLU294, LYS295, TYR299, ARG329, HIS332, and TYR385 were the most stable connections that persisted throughout the MD simulation of Quercetin. In case of Myricetin Important stabilizing interactions include hydrophobic contacts with aromatic residues (TYR, PHE) and hydrogen bonds with polar residues (SER, TYR, GLU, ARG).

The Insilico prediction of lead compound from *R. coriaria* revealed the antiviral potential of myricetin and quercetin based on virtual screening, molecular docking

and MD Simulation.

6.2 Future Recommendations

Based on our research myricetin and quercetin proved to lead compounds. These compounds need further validation before going to drug development against hMPV.

The following further perspective can be addressed.

i. Results confirm the use of myricetin and quercetin as potential compounds for further *in vitro* and *in vivo* studies, as well as new scientific researches for development of novel antiviral agent against hMPV from *R. coriaria*.

ii. Advance drug delivery system such as nano formulations or target carrier system can be further investigated which would be helpful in improving therapeutic efficacy of the compounds.

iii. Examining possible synergistic effects of quercetin and myricetin can be a new objective to be explored in combination with current antiviral drugs for improved treatment consequences.

iv. Furthermore, molecular docking combined with other computational techniques like machine learning and network analysis can be helpful in providing more thorough knowledge of the complex interactions between molecular targets and nutraceuticals.

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