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# Flavonoids as Potent Inhibitor of SARS-CoV-2 Nsp13 Helicase: Grid Based Docking Approach

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**Abstract:** The corona virus (COVID-19) is an enveloped RNA virus with diverse origins in both people and wildlife. It has been determined that six separate species are the cause of human disease. Viral infections have a significant impact on human disease, and one of the most recent worldwide epidemics is the emergence of the new corona. The SS-RNA virus from the enveloped corona virus family is what caused the potentially lethal SARS (Severe Acute Respiratory Syndrome) virus. In many countries throughout the world, sickness is spreading quickly. As of March 26, 2020, there has been 462,684 confirmed cases and 20,834 fatalities documented abroad. COVID-19 was deemed a pandemic by the World Health Organisation (WHO) on March 11, 2020. Numerous drug studies are now underway, and some of the results are positive. The only way to combat the virus, however, is through preventative measures as there is no vaccination. The goal of the current study was to use a molecular docking approach to evaluate flavonoids's potential against SAR-CoV-2 infection. Elucidation of the proposed mechanism of action of natural flavonoid (Quercetin, Isorhametin, Rutin and Tamaraxiten) against SAR-CoV-2 infection.

**Keywords:** SARS-CoV-2 Nsp13 Helicase, Flavonoids, Molecular Docking & Prevention measures.



**Research Paper**

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## INTRODUCTION

A novel corona virus (SARS-CoV) is to blame for the first large worldwide outbreak of the new millennium. Recurrent CoV emergence and outbreaks indicate a concern to the general public's health. It becomes more likely that freshly identified CoVs might spread from human to human as well as from animal to human. The continuous ecological and climatic changes increase the likelihood of future epidemics of these diseases. The COVID-19 corona virus has caused problems in 188 nations and territories worldwide. A study found that the corona virus was to blame for 31 2002 overall cases and 13071 reported fatalities [1]. SARS caused by the corona virus is currently being researched since the optimum therapy is still up for debate. Immune-modulation, supportive care, and antiviral medications [2]. This time, almost ten years after SARS, the Middle East countries have seen the emergence of the extremely dangerous Middle East Respiratory Syndrome Corona virus (MERS-CoV) [3]. The order Nidovirales, which contains the families Coronaviridae, Arteriviridae, and Roniviridae, is mostly comprised of viruses known as corona viruses (CoVs) [4]. A corona virus is an envelope, single-stranded RNA with surface spikes that are between 9 and 12 nm long. Fever, coughing, and shortness of breath are just a few of the symptoms [5-6].

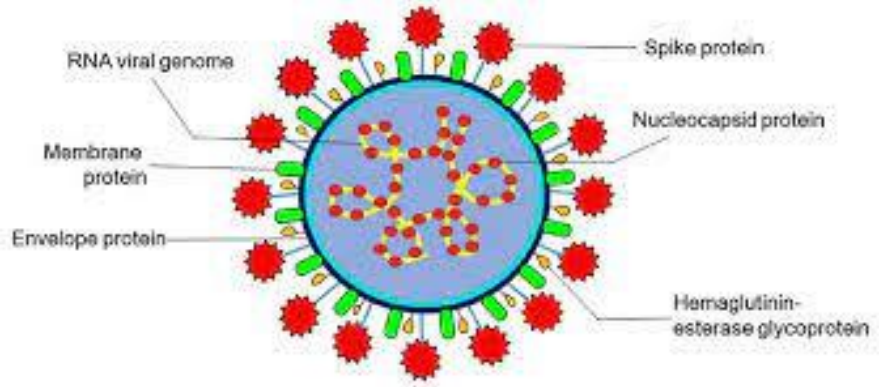



**Figure 1: Structure of CoV**

**Mode of transmission [6]**

- Person-to-person extend of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is thought to occur mainly via respiratory droplets**.**
- Infection can also transpire if a person touches an infected surface and then touches his or her eyes, nose, or mouth.

**Co-morbidity associated with CO-V 19**

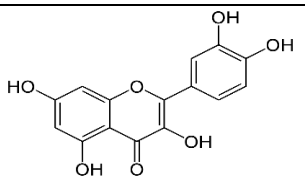
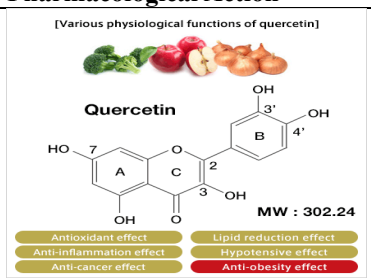
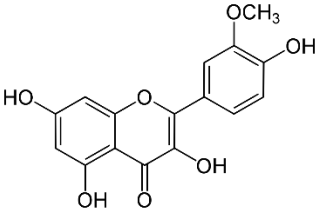
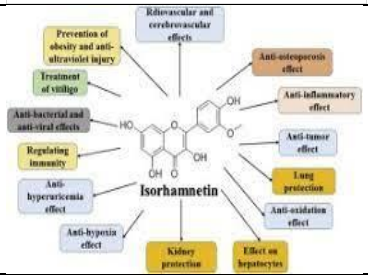
- A logical analysis of CoV cases suggests that diabetes and hypertension are equally ubiquitous in approximately 50% of the patients. CHD are present in 30% and obesity in 16% of the cases. These circumstances down-regulate the synthesis of pro-inflammatory cytokines and impair the host's innate and humoral immune systems [6].

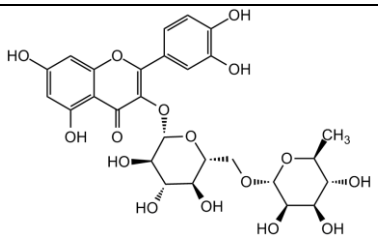
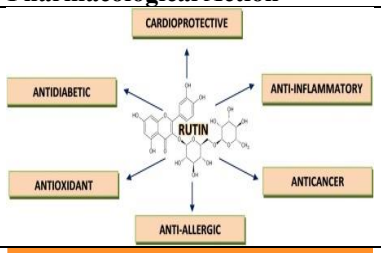
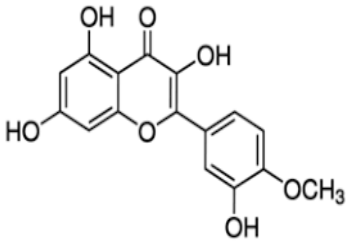
**Prevention of COVID-19 [7]**

- Wash your hands; use a hand sanitizer that contains at least 60% alcohol
- keep away from touching your eyes, nose, and mouth with unwashed hands
- Avoid close contact
- Cover your mouth and nose
- Wear a facemask
- Clean and disinfect frequently touched surfaces daily

Flavonoids, an assembly of natural substances with flexible phenolic structures, are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. These natural products are well known for their beneficial effects on health and efforts are being made to isolate the ingredients so called flavonoids. Flavonoids are now measured as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is accredited to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function.

**Table 1: Selected flavonoids against SAR-CoV-2 infection [8-9]**

| Natural Flavonoids | Structure | Pharmacological Action |
|---|---|---|
| Quercetin |  |  |
| Isorhametin |  |  |

| Natural Flavonoids | Structure | Pharmacological Action |
|---|---|---|
| Rutin |  |   |
| Tamaraxiten |  |   |

The goal of the current study was to use a molecular docking approach to evaluate flavonoids's potential against SAR-CoV-2 infection. Elucidation of the proposed mechanism of action of natural flavonoid (Quercetin, Isorhametin, Rutin and Tamaraxiten) against SAR-CoV-2 infection by targeting *SARS-CoV-2 Nsp13* Helicase.

## EXPERIMENTAL WORK

### Molecular docking studies of SARS-CoV-2 Helicase

***Ligand Preparation:***

2D Structure of ligands like quercetin, rutin, isorhamnetin, and tamarixetin were drawn using ChemSketch [10], the two-dimensional structures of the prepared ligands were converted into their 3-D structures optimized with 3D geometry. The optimized structures were saved in PDB format for AutoDock compatibility [11]. The basic structures of the prepared ligands were given below:

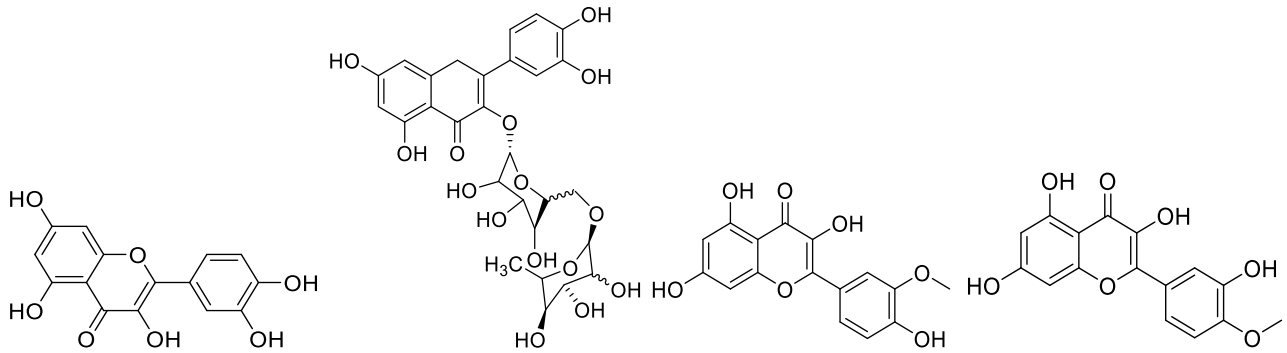


**Figure 2: 2D structure of quercetin, rutin, isorhamnetin, and tamarixetin**

***Preparation of the grid file***

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing between grid points can be adjusted with another thumbwheel, the value in the study taken is 0.458 Å and No. of points considered are 50, 50 and 50 points in the x, y, and z dimensions and -13.606, 25.925 and -70.215 as x, y, z centers [13].

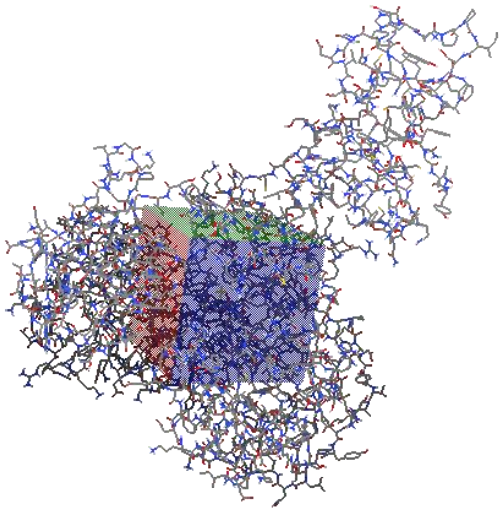


**Figure 3: Grid box covering all active sites in NSP13 helicase enzyme**

***Preparation of the docking file***

All the calculations were carried out by using Autodock 4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [14].

***Crystal structure***

The crystal structure of the protein consisting of NSP13 helicase enzyme is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (6zsl.pdb) registered in the Protein data bank was used [15].

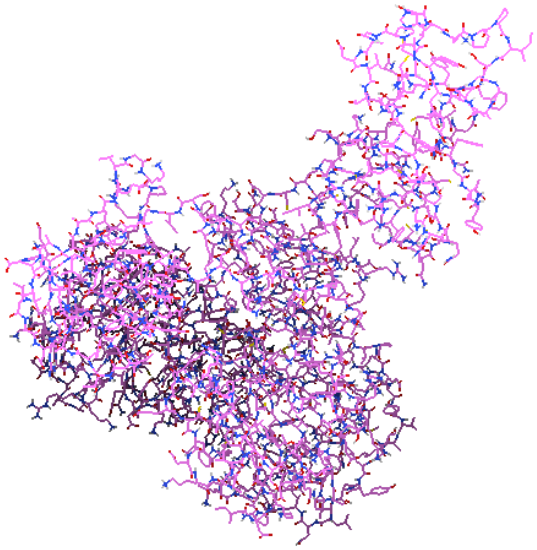


**Figure 4: Crystal structure of NSP13 helicase enzyme (PDB ID-6zsl)**

***Processing of Protein***

The downloaded receptor protein is having two chains, i.e. chain A, and B. Out of these two chains, chain B was selected for experimental purpose and other chains were removed from it. The bound ions were separated from the macromolecular complex by using software Chimera [16].

**Molecular Docking Simulation Studies**

Docking of ligands like quercetin, rutin, isorhamnetin, and tamarixetin against viral NSP13 helicase enzyme was performed by Autodock. All the bonds of each ligand were kept flexible, while no residues in receptor were made flexible [16].

**Toxicity & ADME-T Studies**

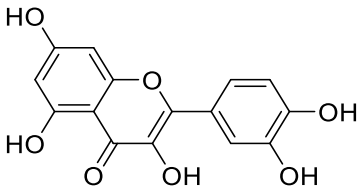
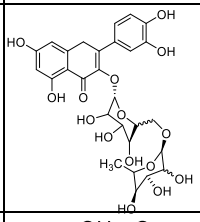
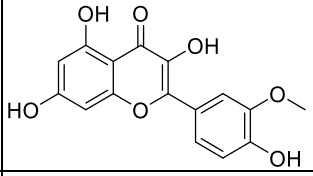
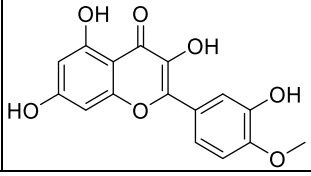
The ligand molecules viz. quercetin, rutin, isorhamnetin, and tamarixetin were studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME-T properties [17].

## RESULTS AND DISCUSSIONS

The new corona virus (SARS-CoV) is the first major epidemic of the new millennium in various countries around the world. CoV epidemics and recurrent epidemics pose a threat to public health. This suggests the possibility of animal-to-human and human-to-human transmission of CoV. Continued changes in ecosystems and climate increase the likelihood of such infections in the future. The new coronavirus, COVID-19, affects 188 countries and territories around the world. A total of 312,002 coronavirus infections and 13,071 deaths have been reported, according to the reported study. Treatment for coronavirus-associated SARS is evolving, and there is no consensus on the optimal treatment regimen. Therapeutic interventions for SARS include broad-spectrum antibiotics and supportive care, antiviral and immunomodulatory therapy. About 10 years after SARS, another highly pathogenic CoV, Middle East respiratory syndrome coronavirus (MERS-CoV), has now appeared in the Middle East, Arteriviridae and Roniviridae. Coronaviruses are enveloped and single-stranded ribonucleic acids with surface spikes up to 9-12 nm in length. Various symptoms include: fever, cough, shortness of breath. Flavonoids, a group of natural substances with a variable phenolic structure, are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. These natural products are well known for their positive health effects, and efforts are being made to isolate the so-called flavonoids. Today, flavonoids are considered an important component in many nutritional, pharmaceutical, medical and cosmetic applications. This is due to their antioxidant, anti-inflammatory, mutagenic and carcinogenic properties, as well as their ability to modulate the functions of important enzymes in the cell.

The immunomodulatory capacity of flavonoids through regulation of inflammatory mediators, inhibition of endothelial activation, activation of NLRP3 inflammasome, toll-like receptors (TLR) or bromodomain-containing protein 4 (BRD4) and nuclear erythro-derived factor 2-related factor 2 (Nrf) . ) may be useful to regulate the cytokine storm in SARS.-during CoV-2 infection. In addition, the ability of flavonoids to inhibit dipeptidyl peptidase 4 (DPP4), neutralize 3-chymotrypsin-like protease (3CLpro) or influence the intestinal microbiota to maintain the immune response and angiotensin-converting enzyme 2 (ACE-2) has a double. effect). could also be used for exaggerated inflammatory responses caused by SARS-CoV-2. Based on previously proven effects of flavonoids on other diseases or recently published studies on COVID-19 by Liskova A *et al;* 2021(18) four flavonoids was selected as lead molecules for current investigation. Moreover, as per literature survey the selected flavonoids showed inhibitory potential against A549 and HCC44 lung cancer cells. So, in current study an attempt had been made to elucidate the proposed mechanism of the action of selected lead compound (flavonoids) by *in -silico* molecular docking.The result of molecular docking was tabulated in table 1, showing binding energy -6.55,-4.65,-7.3,-5.97 kcal/mol for quercetin, rutin, isorhamnetin & tamarixetin respectively. The binding mode showed in fig.4-7 whereas 2D &3D binding interaction was shown in fig.12-19. Although quercetin, rutin & Tamarixetin showed good interaction with selected ligand but highest binding interaction displayed by isorhamnetin with viral NSP13 helicase enzyme having conventional hydrogen bond interaction with Lys A:139, Tyr A:382, Asp A:383, ASN A:381, Arg A:409 Tyr A:120, Pro A:408 as well as Pi- Sigma binding at THR A:380. The pharmacokinetic profiling of the flavonoids ligand has revealed that it is having good pharmacokinetic profile associated without the presence of major toxic effects like reproductive effects, irritant effect, and tumorogenic properties, but shows the presence of some mutagenicity. The pharmacokinetic and toxicity profiling results of flavonoids was shown in fig.8-11.

**Table 2: Result of docking study of Helicase enzyme**

| Sl. No | Compound Name | Structure | Binding |
|---|---|---|---|
| 1 | Quercetin |  | -6.55 |
| 2 | Rutin |  | -4.65 |
| 3 | Isorhamnetin |  | -7.3 |
| 4 | Tamarixetin |  | -5.97 |

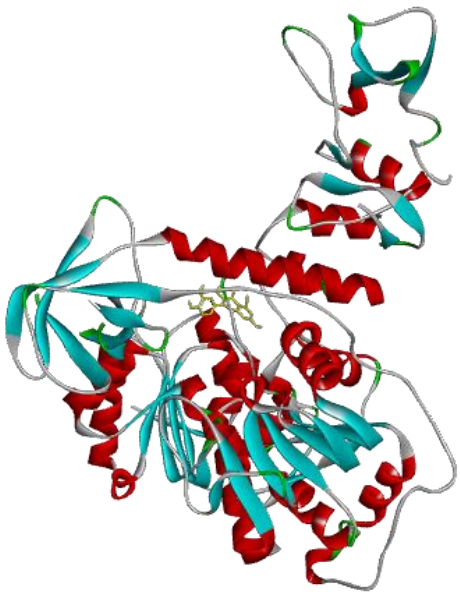


**Figure 5: Binding mode of quercetin within the active site of viral NSP13 helicase enzyme**

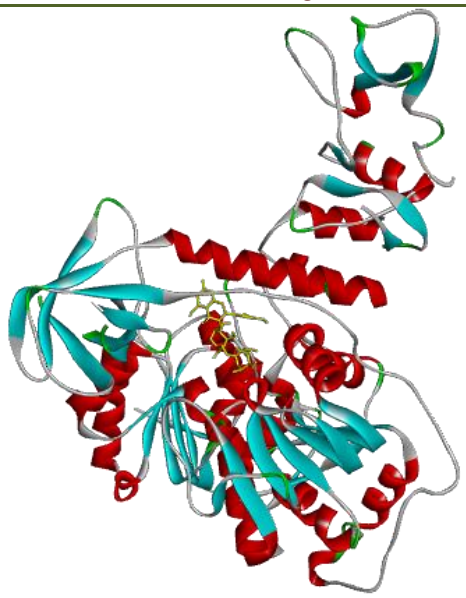


**Figure 6: Binding mode of rutin within the active site of viral NSP13 helicase enzyme**

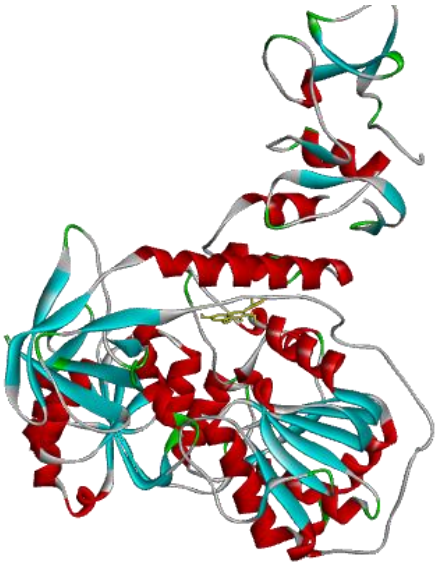


**Figure 7: Binding mode of isorhamnetin within the active site of viral NSP13 helicase enzyme**

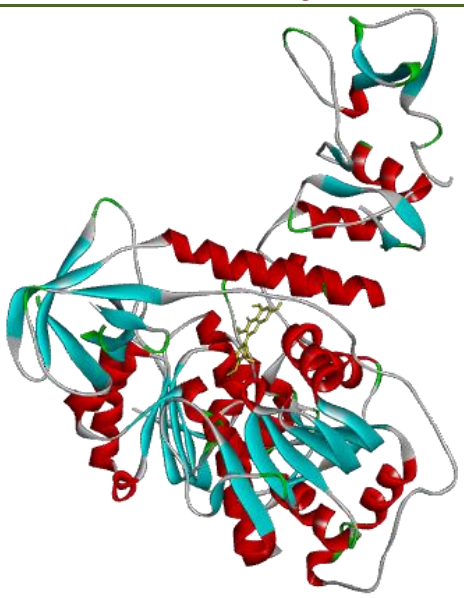


**Figure 8: Binding mode of tamarixetin within the active site of viral NSP13 helicase enzyme**

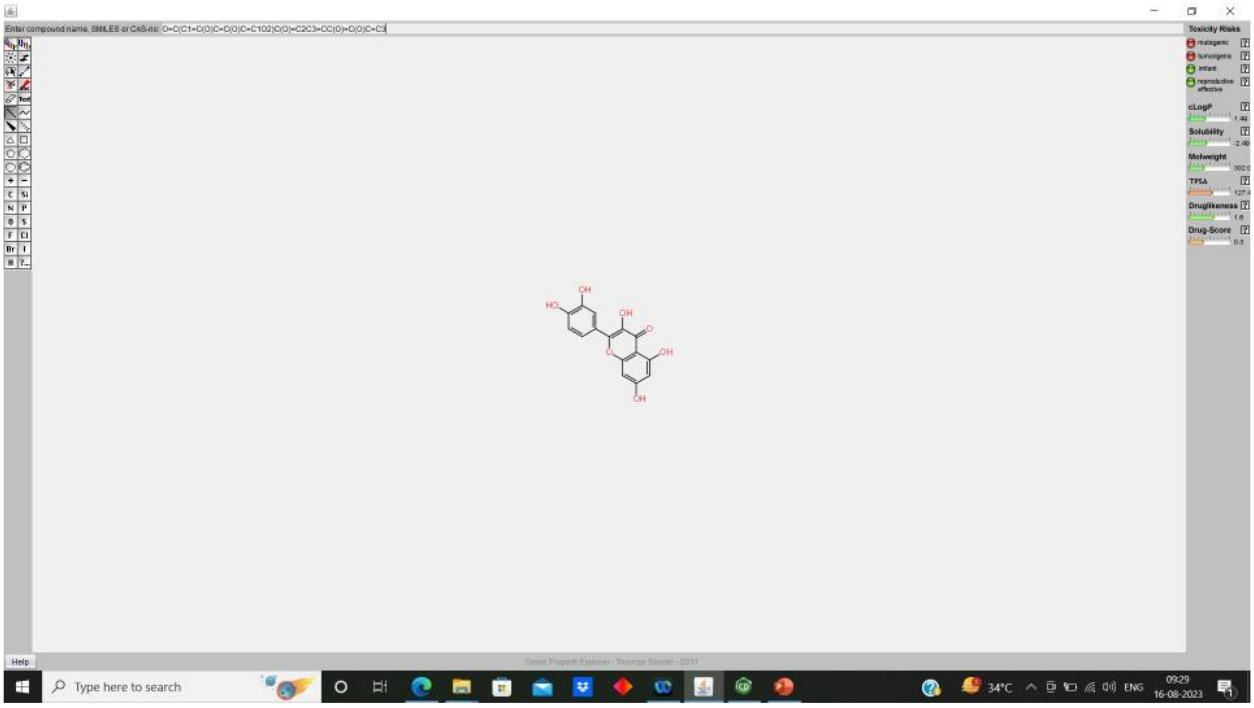



**Figure 9: Pharmacokinetic and toxicity profiling of quercetin**

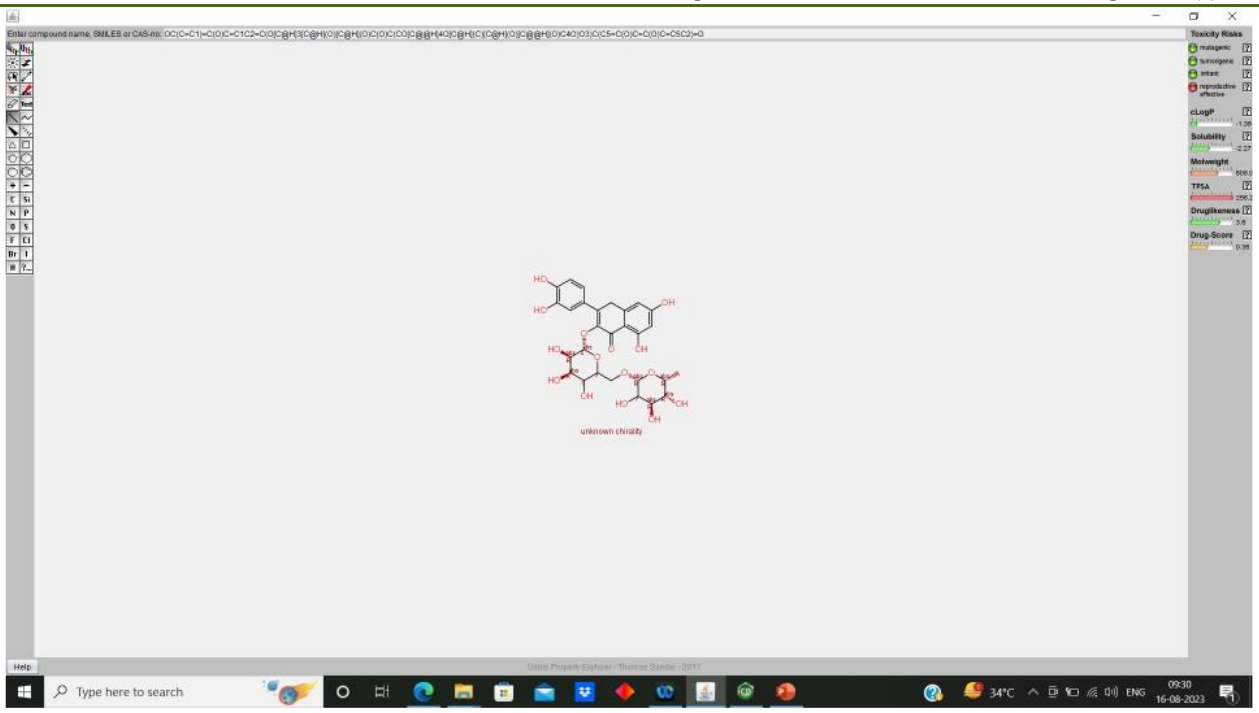



**Figure 10: Pharmacokinetic and toxicity profiling of rutin**

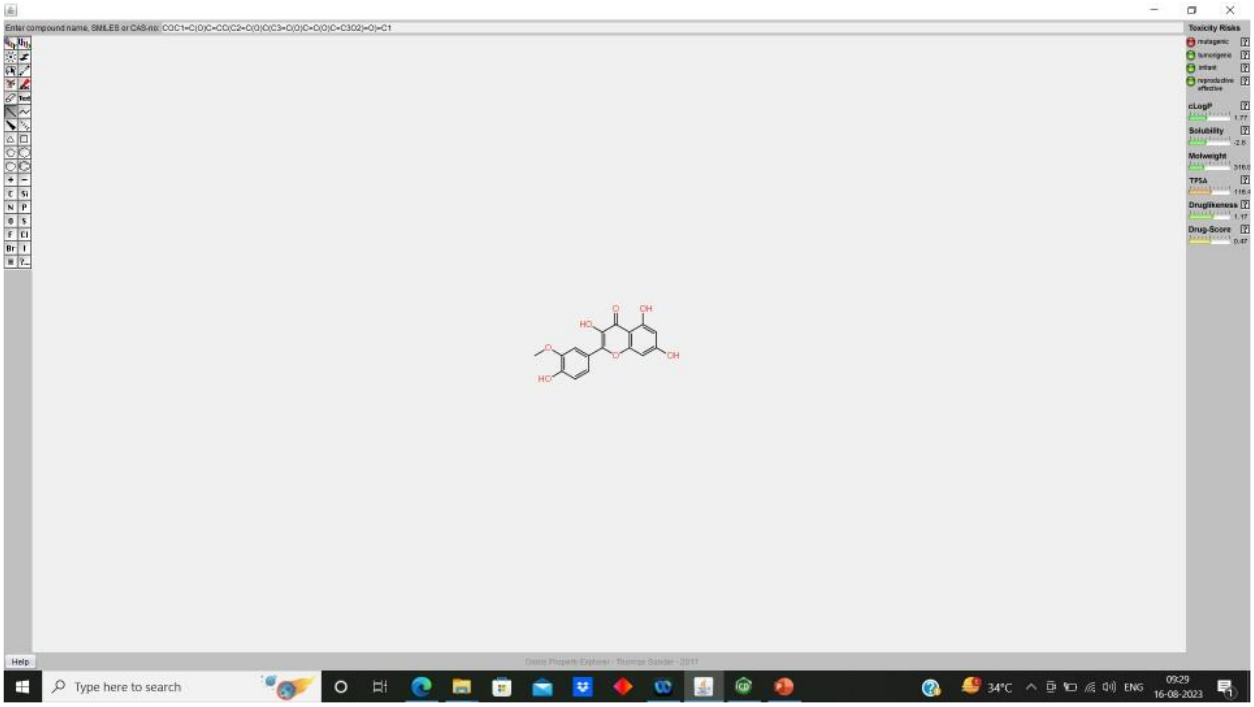



**Figure 11: Pharmacokinetic and toxicity profiling of isorhamnetin**

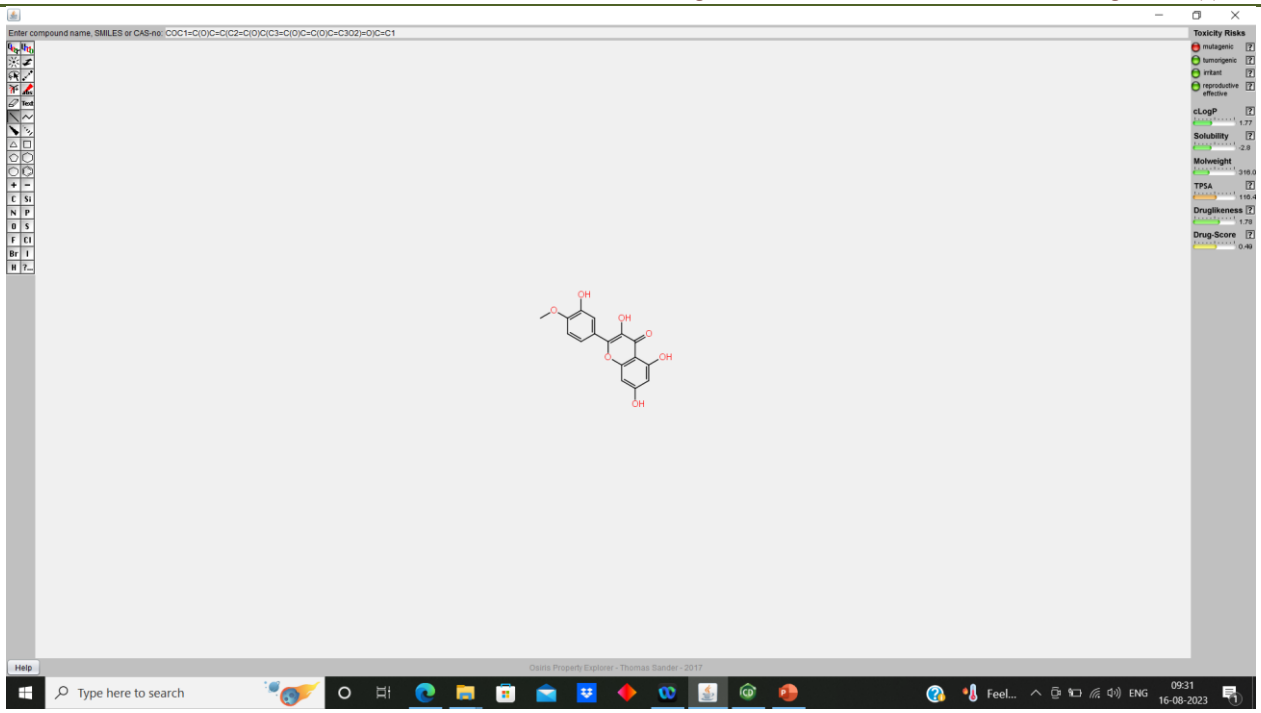



**Figure 12: Pharmacokinetic and toxicity profiling of tamarixetin**

## Interactions

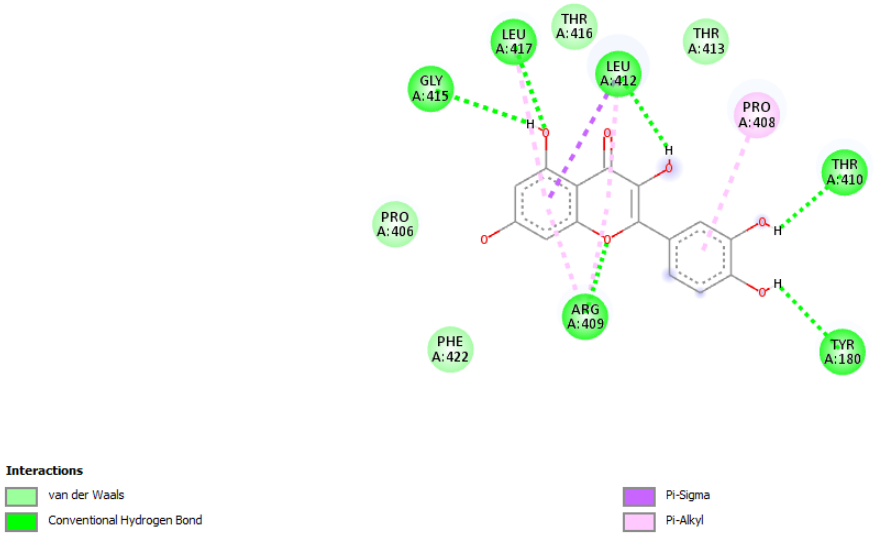



**Figure 13: Two-dimensional binding mode of quercetin within the active site of viral NSP13 helicase enzyme**

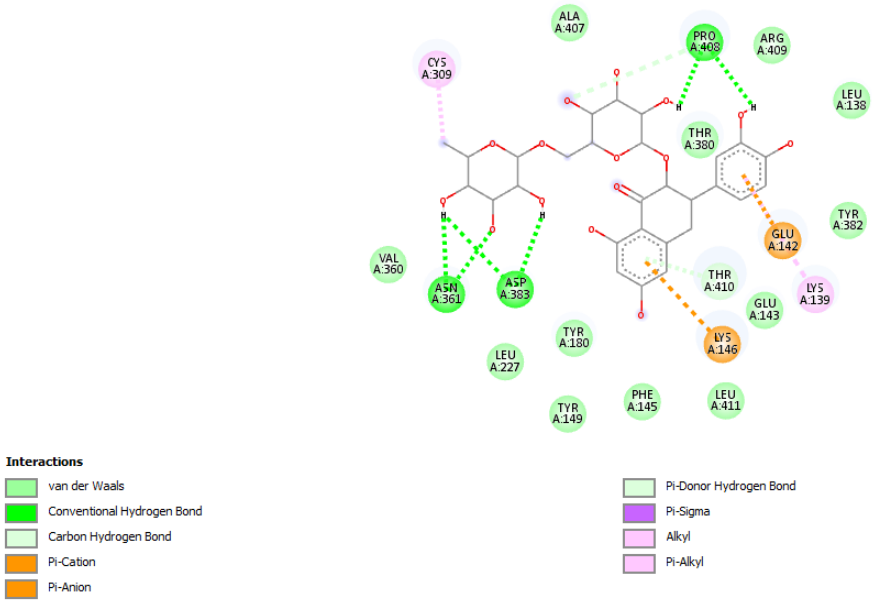



**Figure 14: Two-dimensional binding mode of rutin within the active site of viral NSP13 helicase enzyme**

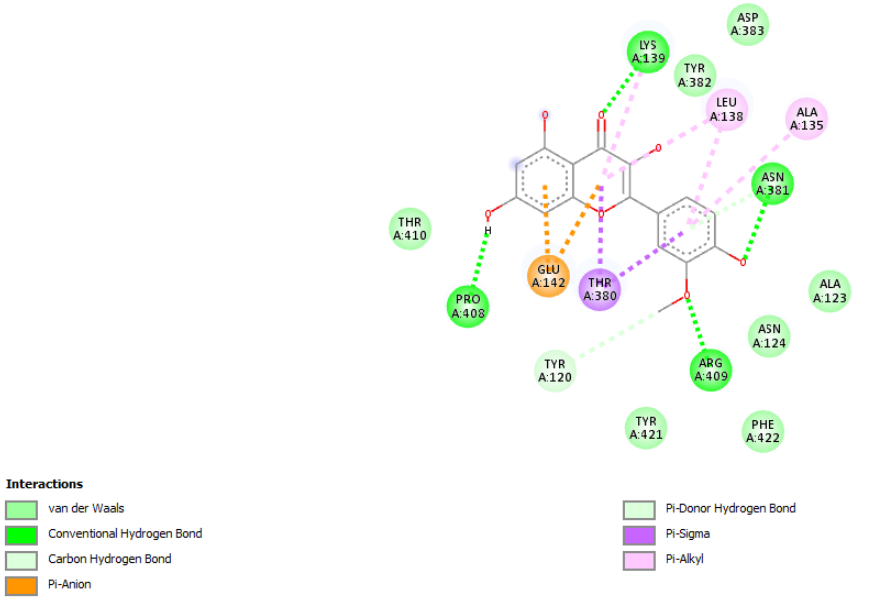



**Figure 15: Two-dimensional binding mode of isorhamnetin within the active site of viral NSP13 helicase enzyme**

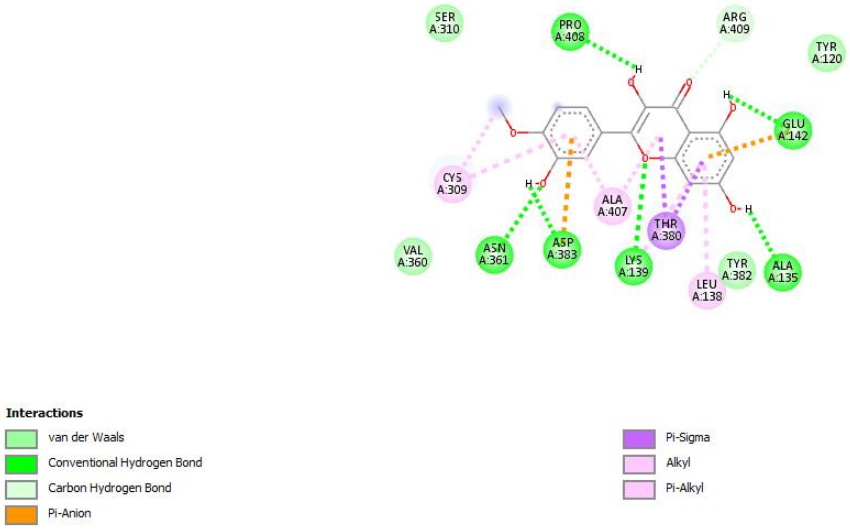



**Figure 16: Two-dimensional binding mode of tamaxiretin within the active site of viral NSP13 helicase enzyme**

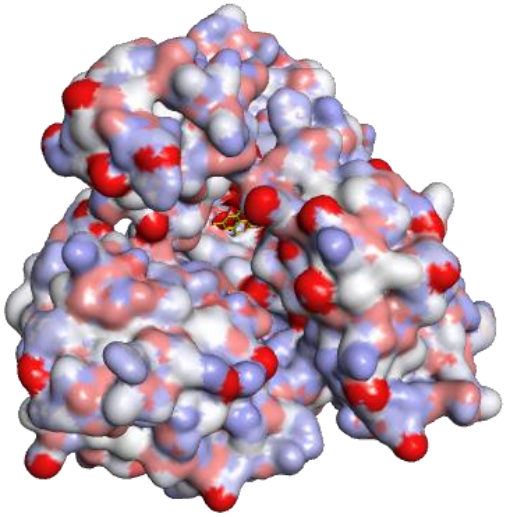


**Figure 17: Three-dimensional binding conformation of quercetin within the active site of viral NSP13 helicase enzyme**

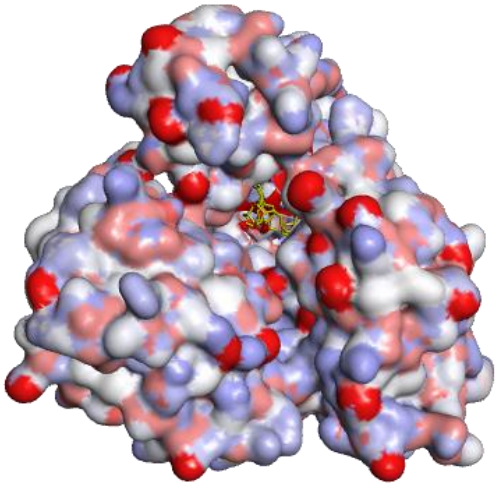


**Figure 18: Three-dimensional binding conformation of rutin within the active site of viral NSP13 helicase enzyme**

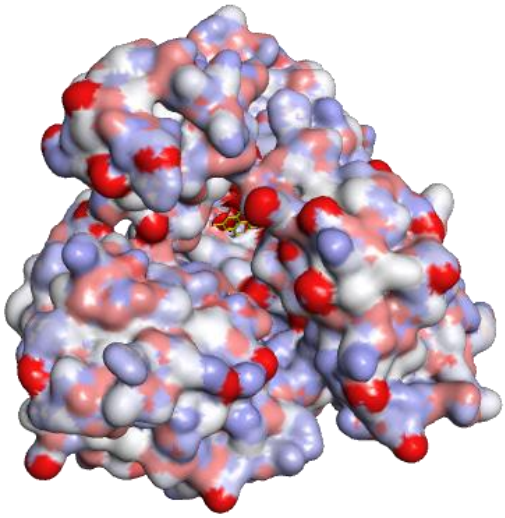


**Figure 19: Three-dimensional binding conformation of isorhamnetin within the active site of viral NSP13 helicase enzyme**

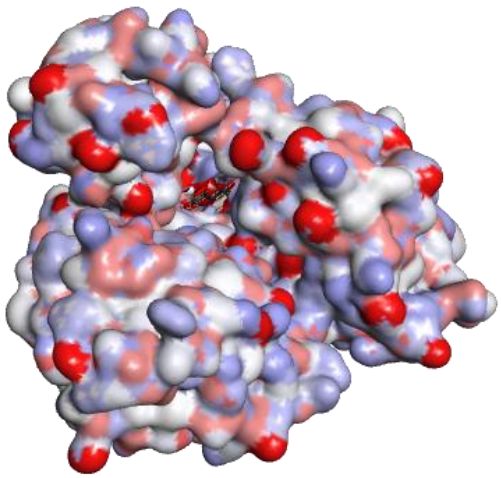


**Figure 20: Three-dimensional binding conformation of tamaxiretin within the active site of viral NSP13 helicase enzyme**

## CONCLUSION

In present work an attempt had been made for assessment of efficacy of flavonoids along with elucidation of proposed mechanism of action against SAR CoV infection. The exact antiviral action of flavonoid against SAR-COV-19 was still not revealed. With intent to propose the most probable mechanism of action of flavonoid the docking based computational analysis has been performed against the antiviral drug targets like SAR CoV Helicase. SARS-CoV-2 helicase *Nsp13* has both ATPase and helicase activity, as it unwinds the RNA helices in an ATP-dependent manner. Remarkably, due to its high sequence conservation across the corona virus family, Nsp13 is considered an attractive target for the development of antiviral drugs. Also, it was shown that SARS-CoV-2 helicase Nsp13 can hydrolyze all types of NTPs including ATP to unwind the RNA helices. Therefore, the known ATP-binding site of the helicase Nsp13 is a promising target for effective inhibition. The outcome of investigation of docking analysis, chemical interactions, followed by the physicochemical based pharmacokinetic profiling has revealed that the flavonoid is executing its antiviral action *via* inhibiting SAR CoV Helicase thereby hindered the ATPase and helicase activity, as it unwinds the RNA helices in an ATP-dependent pattern.The finding revealed that selected flavonoids *i.e.* quercetin, rutin, isorhametin and tamarixetin are potent inhibitor of SARS-CoV-2 helicase *Nsp13* in following manner: isorhametin > quercetin > tamarixetin > rutin.

Theoretically, all the ligand molecules have shown encouraging docking score. The docking result of isorhamnetin revealed that their docking scores was -7.3 kcal $mol^{-1}$, and it can be predicted as good inhibitor of viral ***Nsp13*** helicase enzyme.

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