






## An *in-silico* evaluation of different Saikosaponins for their potency against SARS-CoV-2 using NSP15 and fusion spike glycoprotein as targets

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### ABSTRACT

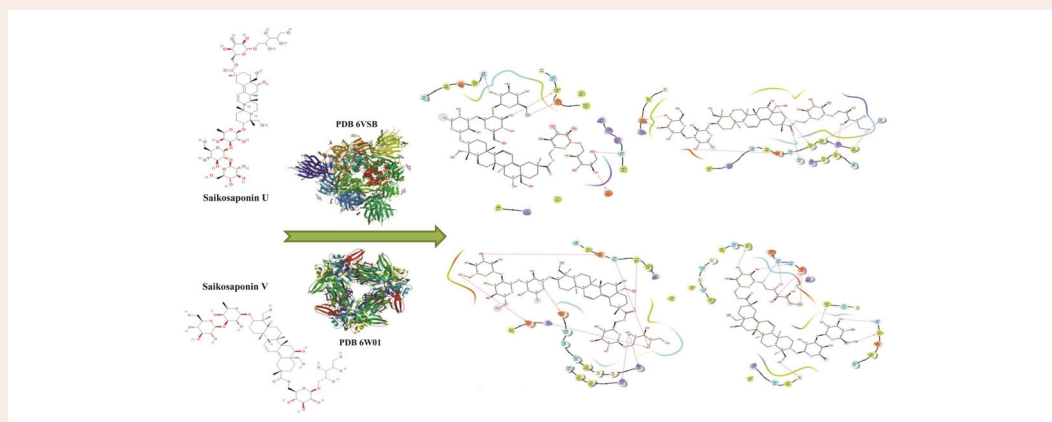
The Public Health Emergency of International Concern declared the widespread outbreak of SARS-CoV-2 as a global pandemic emergency, which has resulted in 1,773,086 confirmed cases including 111,652 human deaths, as on 13 April 2020, as reported to World Health Organization. As of now, there are no vaccines or antiviral drugs declared to be officially useful against the infection. Saikosaponin is a group of oleanane derivatives reported in Chinese medicinal plants and are described for their anti-viral, anti-tumor, anti-inflammatory, anticonvulsant, antinephritis and hepatoprotective activities. They have also been known to have anti-coronaviral property by interfering the early stage of viral replication including absorption and penetration of the virus. Thus, the present study was undertaken to screen and evaluate the potency of different Saikosaponins against different sets of SARS-CoV-2 binding protein *via* computational molecular docking simulations. Docking was carried out on a Glide module of Schrodinger Maestro 2018-1MM Share Version on NSP15 (PDB ID: 6W01) and Prefusion 2019-nCoV spike glycoprotein (PDB ID: 6VSB) from SARS-CoV-2. From the binding energy and interaction studies, the Saikosaponins U and V showed the best affinity towards both the proteins suggesting them to be future research molecule as they mark the desire interaction with NSP15, which is responsible for replication of RNA and also with 2019-nCoV spike glycoprotein which manage the connection with ACE2.

### ARTICLE HISTORY

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### KEYWORDS

Saikosaponins; 2019-nCoV; 6W01; 6VSB; *In-silico*; Corona virus; virtual screening; molecular docking; Traditional Chinese Medicine



**Abbreviations:** ACE2: Angiotensin Converting Enzyme 2; WHO: World Health Organization; SARS: Severe Acute Respiratory syndrome; MERS: Middle East Respiratory Syndrome; SARS-COV-2: Coronavirus Disease Strain; ORF: Open Reading Frame; CoV: Corona Virus; N Protein: Nucleocapsid protein; 2019-nCoV: 2019 Novel Coronavirus; COVID-19: Coronavirus Disease 2019; SDF: Structure Data File; 3CL<sup>Pro</sup>: Chymotrypsin-like protease; TCM: Traditional Chinese Medicine; Th: T helper; IL: Interleukin; IFN: Interferon; TNF: Tumor Necrosis Factor; RBD: Receptor Binding Domain; RMSD: Root Mean Square Deviation; OPLS: Optimized Potentials for Liquid Simulations; NSP: Nonstructural Protein

## Introduction

In the last week of December 2019, Wuhan city, Hubei Province, China emerged with an outbreak of an acute resolved COVID-19 pneumonia, which has subsequently affected around 206 countries around the globe with a 6.1% fatality rate (Xu et al. 2020). The genome sequencing has revealed novel coronavirus SARS-CoV-2, formally known as 2019-nCoV as etiological agent for COVID-19 (Li et al., 2020). The genome of CoVs (enveloped, positive single-stranded RNA) is the largest genome of known RNA viruses with varying size (26 kb–32 kb in length). Coronaviridae family can be divided in four genera comprising of  $\alpha$ - and  $\beta$ -CoV, known to infect mammals, whereas  $\delta$ - and  $\gamma$ - CoVs infect birds. Severe acute respiratory syndrome coronavirus i.e. SARS (2002) and the Middle East respiratory syndrome coronavirus i.e. MERS (2012) were viral pneumonia outbreaks due to  $\beta$ -CoVs and resulted in approximately 800 deaths (Song et al., 2019; Abdo & Eman, 2020). Whereas, this ‘Public Health Emergency of International Concern’ (PHEIC) declared due to widespread outbreak of a SARS-CoV-2 resulted in 1,773,086 confirmed cases of COVID-19, including 111,652 deaths, as of 05:00 pm CEST, 13 April 2020, reported to World Health Organization (WHO) (<https://who.sprinklr.com>) (World Health Organization. 2020).

The SARS-CoV-2 genome (30 kb in size) encodes a large non-structural polyprotein, open reading frame (ORF) 1a/b, which is further proteolytically cleaved to generate 15/16 proteins, five accessory proteins (ORF3a, ORF6, ORF7, ORF8 and ORF9) and four structural proteins (S, M, E and N) deliberated to be essential for SARS-CoV2 assembly and infection (Xu et al., 2020; Ortega et al., 2020; Boopathi et al., 2020).

Protrusion of the CoV spike (S) proteins from the virion envelope to cellular receptors and host cell proteases priming plays a vital role in regulation of entry of coronaviruses into cell. Key atomic-level interactions of the SARS-CoV spike protein receptor-binding domain (RBD) with its host receptor angiotensin-converting enzyme-2 (ACE-2) evidenced the regulation of human-to-human and cross-species transmissions of SARS-CoV (Chen & Du 2020). The recent report on the three-dimensional structure of the crystal structure of the 2019-nCoV main protease (PDB: 6LU7) revealed, that the protein is encoded within the viral genome (Liu et al., 2020), in complex with an inhibitor. Thus, both main protease and spike protein complexed with human ACE-2 receptor might be important targets for researchers to discover and develop vaccines and other therapeutic agents to control this new CoV.

In the absence of any validated approved therapeutic interventions this pandemic is spreading drastically all over the globe. Indeed, in current emergent situation, the concept of drug repositioning might be a cost-effective and time-efficient option for meticulous development of possible therapeutic and/or prophylactic lead candidate(s) from well-known traditional and/or approved drugs (Li et al., 2019). Recent studies comprising reverse vaccinology and via integrated computational approaches have highlighted repurposing of FDA approved drugs and known drugs from various databases against various corona virus enzymes either individually or in combination to combat the virulence in COVID

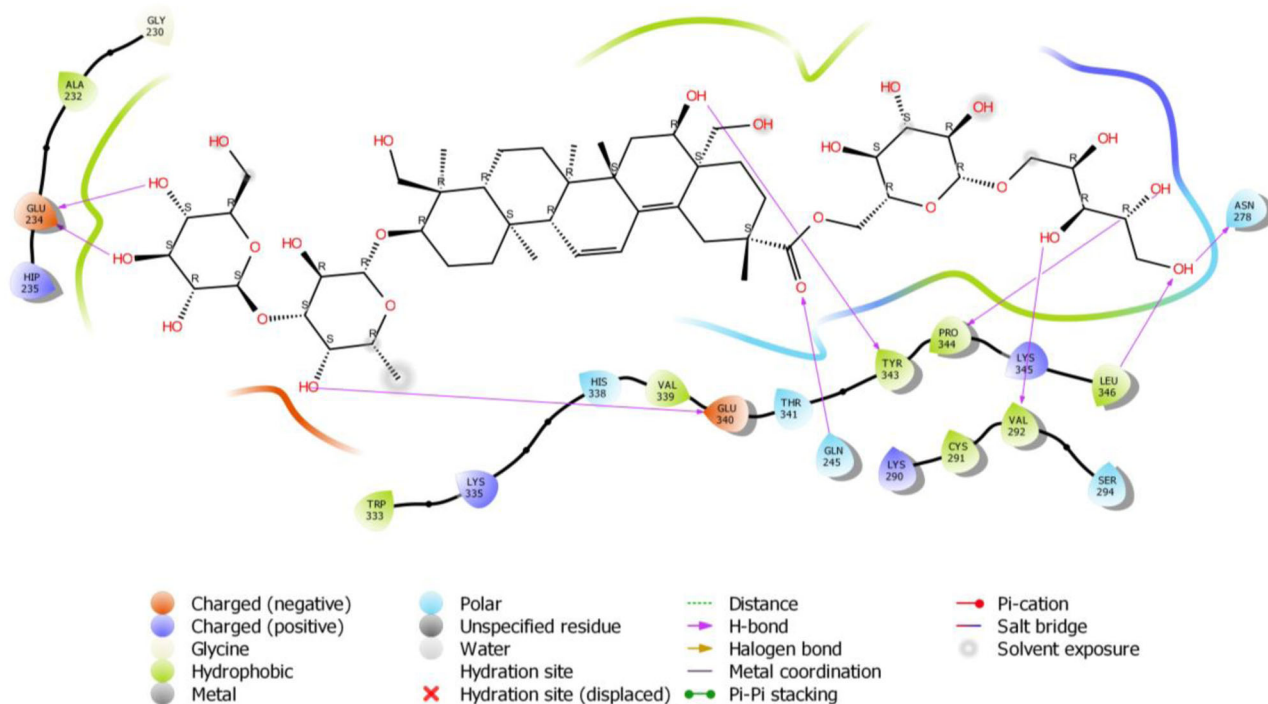
19 (Muralidharan et al., 2020; Elmezayen et al., 2020; Khan et al., 2020; Gupta et al., 2020; Sarma et al., 2020; Pant et al., 2020; Enayatkhani et al. 2020; Khan et al., 2020). The interaction of ACE-2 and with spike protein with furin cleavage site resulting in the invasion of SARS-CoV-2 is also reported (Hasan et al. 2020). Additionally, several phytochemicals were found effective in inhibiting the 3CL<sup>PRO</sup> of SARS-CoV and blocking the entry of SARS-CoV into host cells viz. phenolic compounds of *Isatis indigotica* root (Lin et al., 2005), baicalin (Chen et al., 2004), glycyrrhizin (Cinatl et al., 2003), quercetin (Chen et al., 2006) and Saikosaponin (Cheng et al., 2006). Moreover, ‘leads’ from natural products has also been explored for their efficacy against SARS-CoV-2 (Aanouz et al., 2020), and hesperetin, a natural citrus fruit flavonoid is found promising in suppressing the cleavage activity of the 3C-like protease (3CL<sup>PRO</sup>) of SARS-CoV in cell-free and cell-based assays (Chen et al., 2020).

Saikosaponins represent a group of oleanane derivatives, usually glucosides and are major bioactive compounds found in plants of Traditional Chinese Medicine (TCM) such as *Bupleurum spp.*, *Heteromorpha spp.* and *Scrophularia scorodonia* (Li et al. 2018a; Li et al., 2018b). Saikosaponins have been reported for various biological activities like anti-viral, anti-tumor, anti-inflammatory, anticonvulsant, antinephritis and hepatoprotective activity both *in-vivo* and *in-vitro* (Chen et al., 2015; Yang et al., 2017; Xiao et al., 2018). Saikosaponins exhibit immunomodulatory effects with enhanced production of immunosuppressive mediators i.e. T helper (Th) 1/Th2 cells, IL-4 and IL-10 and suppression of pro-immune mediators (IFN- $\gamma$  and TNF- $\alpha$  in splenocytes) (Huang et al., 2017). Inhibitory potential of Saikosaponins for HBV-DNA replication and HBsAg secretion, without inhibition of cell proliferation has been found to be superior to lamivudine (well-known antiviral drug) (Lin et al., 2015). Moreover, Saikosaponins (A, B<sub>2</sub>, C and D) have also been reported for their anti-coronaviral efficacy by interfering the early stage of viral replication including absorption and penetration of the virus (Cheng et al., 2006). A recent study, performed by Goswami and Bagehi (2020) reported the potential of Saikosaponins against SARS-CoV-2, showing favorable affinity towards RBD region of the spike glycoprotein using docking simulation study (Goswami & Bagchi 2020). However, it will be interesting to investigate the potency of different class of Saikosaponins towards the other protein such as NSP15 and fusion spike (S1 and S2) protein, which will give a clear indication about the selectivity of the Saikosaponins towards SARS-CoV-2. Therefore, in the current investigation attempt has been made to screen and evaluate the potency of different Saikosaponins against different sets of SARS-CoV-2 binding protein *via* computational molecular docking simulations.

## Material and methods

### Protein preparation

The docking simulation study of total 23 Saikosaponins (Supplementary file) was performed on the 1.9A crystal structure of NSP15 Endoribonuclease from SARS CoV-2 in the complex with a citrate (PDB ID: 6W01) and prefusion 2019-



**Figure 1.** Binding-interaction analysis of Saikosaponin V with NSP15 endoribonuclease (PDB ID: 6W01).

nCoV spike glycoprotein with a single receptor-binding domain up (PDB ID: 6VSB) which was retrieved from protein data bank (<https://www.rcsb.org>). The protein preparation wizard of Schrödinger maestro 2018-1 MM share version was used to prepare the structure by adding hydrogen, treating metal, deleting water molecules and assigning partial charges by using the OPLS-2005 force field, then assigning protonation states and determining restrained and further partial energy was minimized with 0.3 Å RMSD limit. The binding sites were defined after removing the ligand then grid was generated using a grid box volume of 10\_10\_10 Å.

### Ligand preparation

The 2D structure of ligands were downloaded from pubchem compound database (<https://pubchem.ncbi.nlm.nih.gov>) in SDF format and uploaded to the work space. Then ligprep module of maestro was used to generate the 3D structure and OPLS-2005 force field was used to generate the different conformation of each ligand. The stable conformer of each Saikosaponin with minimum potential energy was further processed.

### Protein ligand docking

Glide module of Schrödinger suite was used to dock the each ligand into the identified binding site of grid, and the lowest binding pose of each docking run was retained. The results of simulations were analyzed using glide XP visualizer, and the important active site interactions were analyzed along with the scoring functions.

## Results

To check the potency of different Saikosaponins, the first docking simulation study was done on the crystal structure of NSP15 Endoribonuclease from SARS CoV-2 (PDB Code: 6W01). The results revealed that Saikosaponin V was properly positioned into the binding pocket constructed by polar Asn 278, Thr 341, Ser 294, Gln 245 His 338, hydrophobic Trp 333, Pro 344, Gly 230, Leu 346, Tyr 343, Val 339, Ala 232, Cys 291, Val 292 charged Glu 340, Lys 345, Lys 335, Lys 290, Hip 235 and Gln 245 amino acids with binding energy -8.35 Kcal/mol (Figure 1). Saikosaponin compounds with binding interaction parameter i.e. binding energy with the PDB: **6W01** and **6VSB** of SARS-CoV-2 is depicted in Table 1 whereas details of binding interactions of top five Saikosaponins against both protein targets comprising details of protein residues involved in hydrogen bonding and hydrophobic interactions are summarized in Tables 2 and 3.

Among them, Glu 234 exhibited bi-hedral H-bonding with hydroxyl group of oxane ring (tetrahydropyran) of Saikosaponin V and other hydroxyl group of Saikosaponin V showed H-bonding with Asn 278, Pro 344, Val 292, Tyr 343 and Leu 346. Hydroxyl group of oxane ring of Saikosaponin V showed H-bonding with Glu 340 and carboxyl group of Saikosaponin V showed H-bonding with Glu 245.

Saikosaponin U was found to interact with the binding pocket constructed by polar Ser 294, Thr 341, Ser 242, His 338, His 243, Gln 245 hydrophobic Val 339, Leu 246, Pro 344, Tyr 343, Trp 333, Val 292, Cys 293, Ala 232 charged Lys 345, Glu 234, Asp 240, Glu 340, Hip 250, Lys 290 and Hip 235 amino acids with binding energy -7.27 Kcal/mol. Hydroxyl group of oxane ring of Saikosaponin U showed H-bonding with Asp 240 and Glu340 (Figure 2).

**Table 1.** List of compounds with binding interaction parameter i.e. binding energy with the PDB: **6W01** and **6VSB** of SARS-CoV-2.

Sr. No.	Pubchem CID	Name	Binding energy Kcal/mol	
			(6W01)	(6VSB)
1	100958093	Saikosaponin V	-8.358	-8.299
2	100958092	Saikosaponin U	-7.272	-8.429
3	46783811	Saikosaponin C	-6.981	-7.274
4	100962152	Saikosaponin K	-6.79	-6.251
5	124222337	Saikosaponin 1b	-6.367	-6.195
6	100962153	Saikosaponin N	-6.192	-6.142
7	132533303	Saikosaponin R	-6.139	-6.615
8	10534262	Saikosaponin O	-5.783	-5.538
9	100962151	Saikosaponin Q	-5.728	-5.518
10	101826550	Saikosaponin G	-5.663	-5.695
11	21637636	Saikosaponin B4	-5.587	-5.808
12	21636280	Saikosaponin H	-5.531	-4.775
13	21637635	Saikosaponin B3	-5.175	-6.19
14	102316649	Saikosaponin 2	-4.955	-4.085
15	441945	Saikosaponin BK1	-4.887	-6.195
16	167928	Saikosaponin A	-4.725	-5.143
17	21637642	Saikosaponin B2	-4.697	-6.19
18	9875547	Saikosaponin B1	-4.61	-6.096
19	21598300	Saikosaponin F	-4.396	-5.529
20	21636282	Saikosaponin I	-4.3	-5.794
21	102123130	16-Keto Saikosaponin A	-3.973	-5.301
22	21637632	Saikosaponin E	-3.576	-4.905
23	107793	Saikosaponin D	-3.738	-5.638

**Table 2.** Binding interactions of different Saikosaponins with the active site of SARS-CoV-2 NSP15 endoribonuclease (PDB ID: **6W01**).

Sr. No.	Ligand	Interaction (PDB-6W01)	
		H-Bonding	Hydrophobic Pocket
1	<b>Saikosaponin V</b>	Glu 234, Asn 278, Pro 344, Val 292, Tyr 343, Leu 346, Glu 340, Gln 245	Trp 333, Pro 344, Gly 230, Leu 346, Tyr 343, Val 339, Ala 232, Cys 291, Val 292
2	<b>Saikosaponin U</b>	Asp 240, Hip 250, Lys 290, Hip 235, Thr 341, Glu 234, Tyr 343, Glu 340	Val 339, Leu 246, Pro 344, Tyr 343, Trp 333, Val 292, Cys 293, Ala 232
3	<b>Saikosaponin C</b>	Lys 290, Gln 245, Hip 235, Thr 341, Asn 278, Leu 346	Val 292, Leu 346, Pro 344, Tyr 343, Trp 333, Cys 293, Leu 246, Gly 247, Gly 248
4	<b>Saikosaponin K</b>	Val 292, Hip 250, Gly 248, Tyr 343, Glu 340	Val 292, Leu 246, Tyr 343, Trp 333, Cys 293, Gly 247, Gly 248, Cys 291, Met 331, Ala 232
5	<b>Saikosaponin 1b</b>	Gly 245, Gln 245, Glu 234, Gly 230	Val 339, Leu 246, Tyr 343, Trp 333, Gly 247, Gly 248, Ala 232, Gly 230

**Table 3.** Binding interactions of different Saikosaponins with the active site of SARS-CoV-2 spike glycoprotein (PDB ID: **6VSB**).

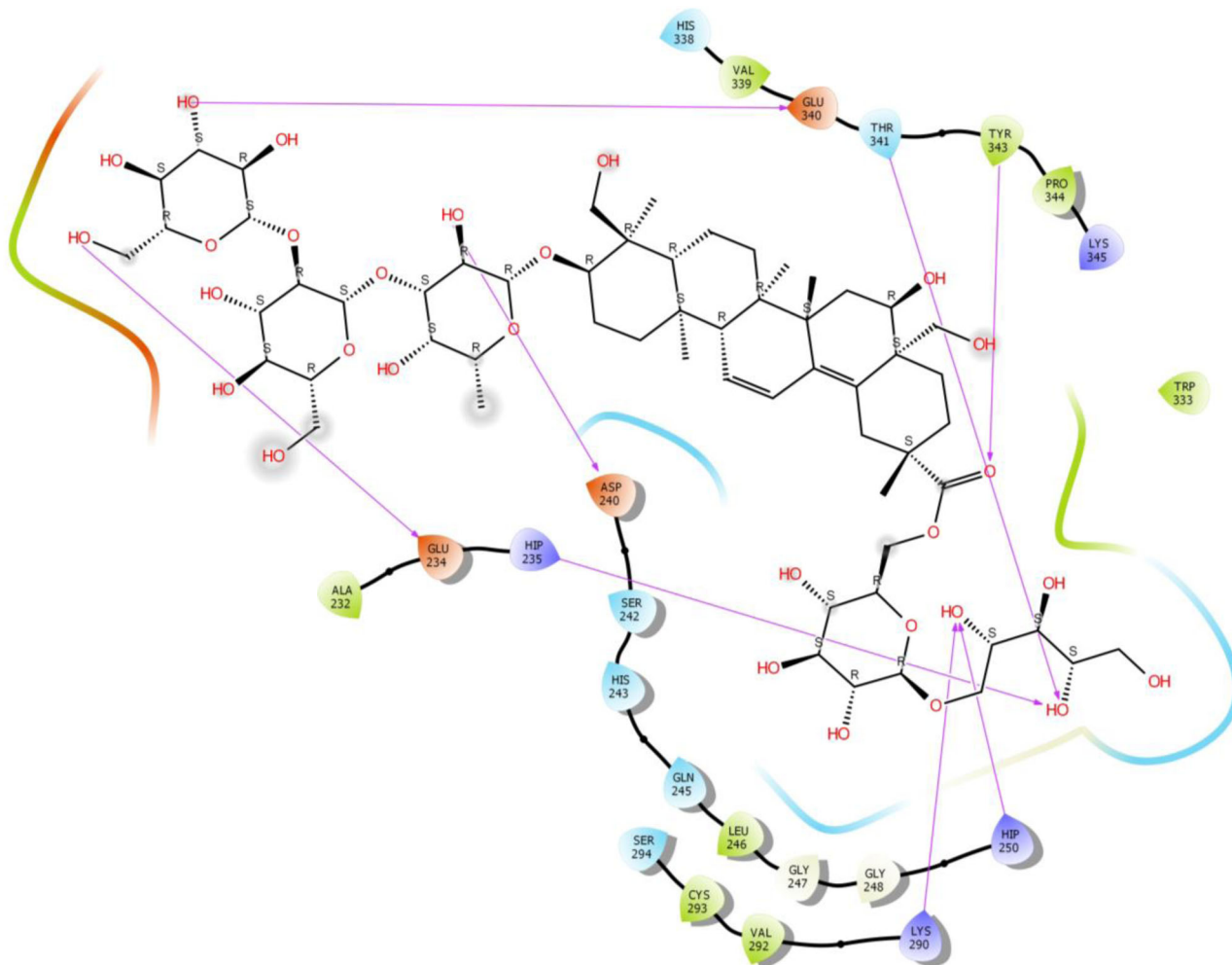
Sr. No.	Ligand	Interaction (PDB-6VSB)	
		H-Bonding	Hydrophobic Pocket
1	<b>Saikosaponin U</b>	Phe 346, Ser 402, Asn 271, Glu 257, Asp 345	Trp 270, Pro 343, Phe 346, Gly 348, Phe 403, Leu 405, Phe 371, Tyr 313
2	<b>Saikosaponin V</b>	Phe 346, Ser 402, Thr 460, Ile 475, Asp 459, Thr 461, Phe 403, Gly 298	Val 299, Leu 307, Phe 346, Phe 403, Leu 405, Phe 429, Leu 434, Ile 475, Pro 477
3	<b>Saikosaponin C</b>	Phe 403, Asp 345, Lys 341, Glu 372	Val 299, Tyr 313, Tyr 338, Leu 342, Pro 343, Phe 346, Pro 370, Phe 371, Phe 403, Leu 405
4	<b>Saikosaponin K</b>	Phe 403, Ser 402, Glu 404, Asp 345	Tyr 313, Leu 342, Pro 343, Phe 346, Pro 370, Phe 371, Phe 403, Leu 405
5	<b>Saikosaponin R</b>	Thr 460, Ile 475, Ala 458, Thr 435	Val 299, Leu 304, Leu 307, Leu 405, Leu 434, Phe 346, 429, Ile 475, Pro 477, Tyr 497, Gly 298, Ala 458, Ile 457

The other hydroxyl group of Saikosaponin U showed H-bonding with Hip 250, Lys 290, Hip 235 and Thr 341. The hydroxymethyl group of oxane ring of Saikosaponin U showed H-bonding with Glu 234 and carboxyl group of Saikosaponin U showed H-bonding with Tyr 343.

Saikosaponin C was properly positioned into the binding pocket constructed by polar Ser 244, Thr 341, Ser 294, His 243, Gln 245, Asn 278, hydrophobic Val 292, Leu 346, Pro 344, Tyr 343, Trp 333, Cys 293, Leu 246, Gly 247, Gly 248

charged Lys 345, Asp 240, Hip 250, Lys 290 and Hip 235 amino acids with binding energy -6.98 Kcal/mol. The hydroxyl group of oxane ring of Saikosaponin C exhibited bi-hedral H-bonding with Lys 290 and other hydroxyl group of oxane ring of Saikosaponin C showed H-bonding with Hip 235, Thr 341 and Asn 278 and tetrahydrofuran ring of Saikosaponin C showed H-bonding with Gln 245 (Figure 3).

Saikosaponin K was found to interact with the binding pocket constructed by polar Ser 294, Thr 341, His 338, Gln



**Figure 2.** Binding-interaction analysis of Saikosaponin U with NSP15 endoribonuclease (PDB ID: 6W01).

245, hydrophobic Val 292, Leu 246, Tyr 343, Trp 333, Cys 293, Gly 247, Gly 248, Cys 291, Met 331, Ala 232 charged Lys 345, Hip 250, Lys 290, Lys 335, Glu 340 and Hip 235 amino acids with binding energy -6.79 Kcal/mol. Hydroxyl group of oxane ring of Saikosaponin K showed H-bonding with Val 292, Hip 250, Gly 248 while, hydroxymethyl group of oxane ring of Saikosaponin K showed H-bonding with Tyr 343 and other with Glu 340 (Figure 4).

Saikosaponin 1 b was properly positioned into the binding pocket constructed by polar Thr 341, His 338, Gln 245, His 243, hydrophobic Val 339, Leu 246, Tyr 343, Trp 333, Gly 247, Gly 248, Ala 232, Gly 230 charged Lys 290, Hip 250, Glu 340, Asp 240, Glu 234, Glu 229 and Hip 235 amino acids with binding energy -6.36 Kcal/mol.

Among them, Gly 245 exhibited bi-hedral H-bonding with hydroxyl group of oxane ring of Saikosaponin 1 b and other hydroxyl group of Saikosaponin 1 b Glu 234. Hydroxymethyl group of oxane ring of Saikosaponin 1 b showed H-bonding with Gln 245 and other hydroxymethyl group with Glu 234 and Gly 230 (Figure 5).

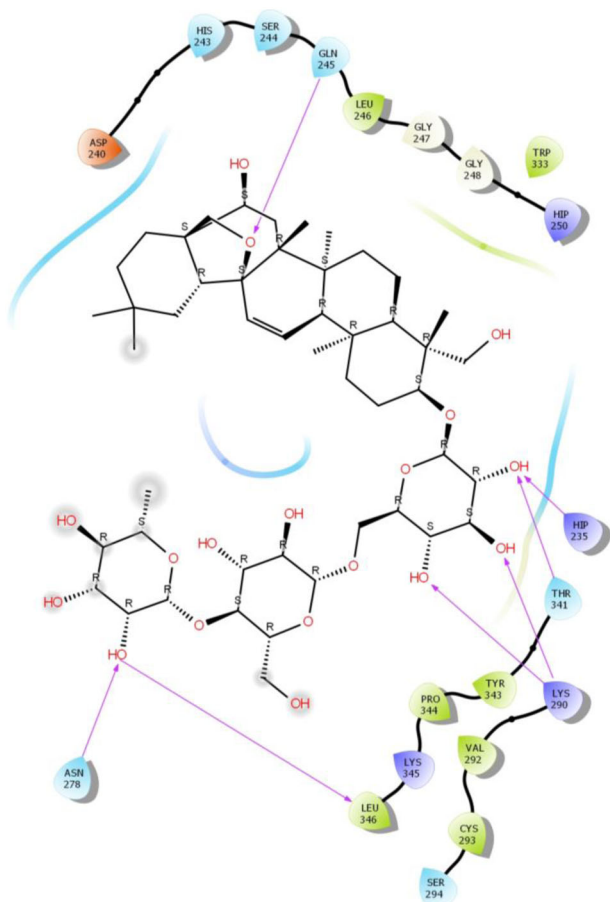
The second study was performed on the crystal structure of COVID-19 main protease enzyme (PDB Code: 6VSB). The results revealed that Saikosaponin U was properly positioned into the binding pocket constructed by polar Asn 271, Thr 347, Ser 402, His 407, hydrophobic Trp 270, Pro 343, Phe

346, Gly 348, Phe 403, Leu 405, Phe 371, Tyr 313 charged Glu 257, Arg 272, Lys 273, Arg 274, Asp 345, Arg 373 and Glu 404 amino acids with binding energy -8.42 Kcal/mol.

Hydroxyl group of oxane ring of Saikosaponin U showed H-bonding with Phe 346, Ser 402 and other hydroxyl group of Saikosaponin U showed H-bonding with Asn 271 and Glu 257, Hydroxymethyl group of oxane ring of Saikosaponin U showed H-bonding with Asp 345 (Figure 6).

Saikosaponin V was found to interact with the binding pocket constructed by polar Ser 300, Thr 347, Ser 402, His 407, Thr 435, Thr 437, Thr 460, Thr 461, Thr 476 hydrophobic Val 299, Leu 307, Phe 346, Phe 403, Leu 405, Phe 429, Leu 434, Ile 475, Pro 477 charged Lys 303, Glu 404, Arg 455, Asp 459 and Asp 462 amino acids with binding energy -8.29 Kcal/mol. Phe 346, Ser 402, Thr 460, Ile 475 exhibited H-bonding with Hydroxyl group of oxane ring of Saikosaponin V and other hydroxyl group of Saikosaponin V showed H-bonding with Asp 459 and Thr 461, Hydroxymethyl group of oxane ring of Saikosaponin V showed H-bonding with Phe 403, Hydroxymethyl group of other ring showed H-bonding with Gly 298 and ether linkage showed H-bonding with Thr 461 (Figure 7).

Saikosaponin C was properly positioned into the binding pocket constructed by polar Thr 347, Ser 402, His 407, hydrophobic Val 299, Tyr 313, Tyr 338, Leu 342, Pro 343, Phe 346,



**Figure 3.** Binding-interaction analysis of Saikosaponin C with NSP15 endoribonuclease (PDB ID: 6W01).

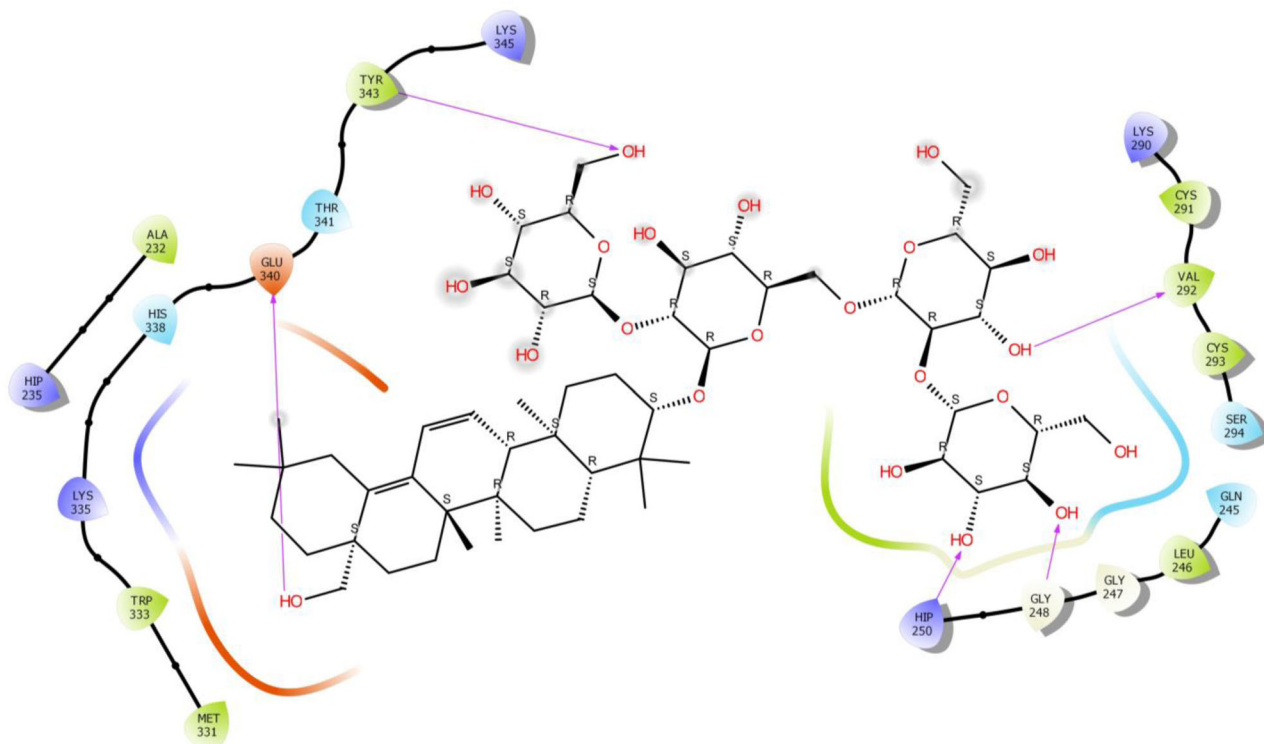
Pro 370, Phe 371, Phe 403, Leu 405 charged Lys 341, Asp 344, Asp 345, Lys 369, Glu 372, Arg 373 and Glu 404 amino acids with binding energy  $-7.27$  Kcal/mol. Hydroxyl group of oxane ring of Saikosaponin C showed H-bonding with Phe 403 and Asp 345, other hydroxyl group of Saikosaponin C showed H-bonding with Lys 341 and Glu 372 (Figure 8).

Saikosaponin K was found to interact with the binding pocket constructed by polar Asn 311, Thr 347, Ser 402, His 407 hydrophobic Tyr 313, Leu 342, Pro 343, Phe 346, Pro 370, Phe 371, Phe 403, Leu 405 charged Lys 341, Asp 345, Lys 369, Glu 372 and Glu 404 amino acids with binding energy  $-6.25$  Kcal/mol. The hydroxyl group of oxane ring of Saikosaponin K exhibited H-bonding with Phe 403, Ser 402, Glu 404. Hydroxymethyl group of oxane ring of Saikosaponin V showed H-bonding with Asp 345 (Figure 9).

Saikosaponin R was properly positioned into the binding pocket constructed by polar Ser 300, Thr 435, Thr 460, Thr 461, Thr 476, hydrophobic Val 299, Leu 304, Leu 307, Leu 405, Leu 434, Phe 346, 429, Ile 475, Pro 477, Tyr 497, Gly 298, Ala 458, Ile 457 charged Lys 303, Asp 459 and Asp 462 amino acids with binding energy  $-6.19$  Kcal/mol. Hydroxyl group of oxane ring of Saikosaponin R showed H-bonding with Thr 460, Ile 475 and bi-hedral H-bonding with Ala 458, hydroxymethyl group of oxane ring of Saikosaponin R showed H-bonding with Thr 435 (Figure 10).

## Discussion

The corona viruses are said to be enveloped RNA viruses having the largest genome among all RNA viruses and containing a replicase gene covered with nonstructural proteins



**Figure 4.** Binding-interaction analysis of Saikosaponin K with NSP15 endoribonuclease (PDB ID: 6W01).

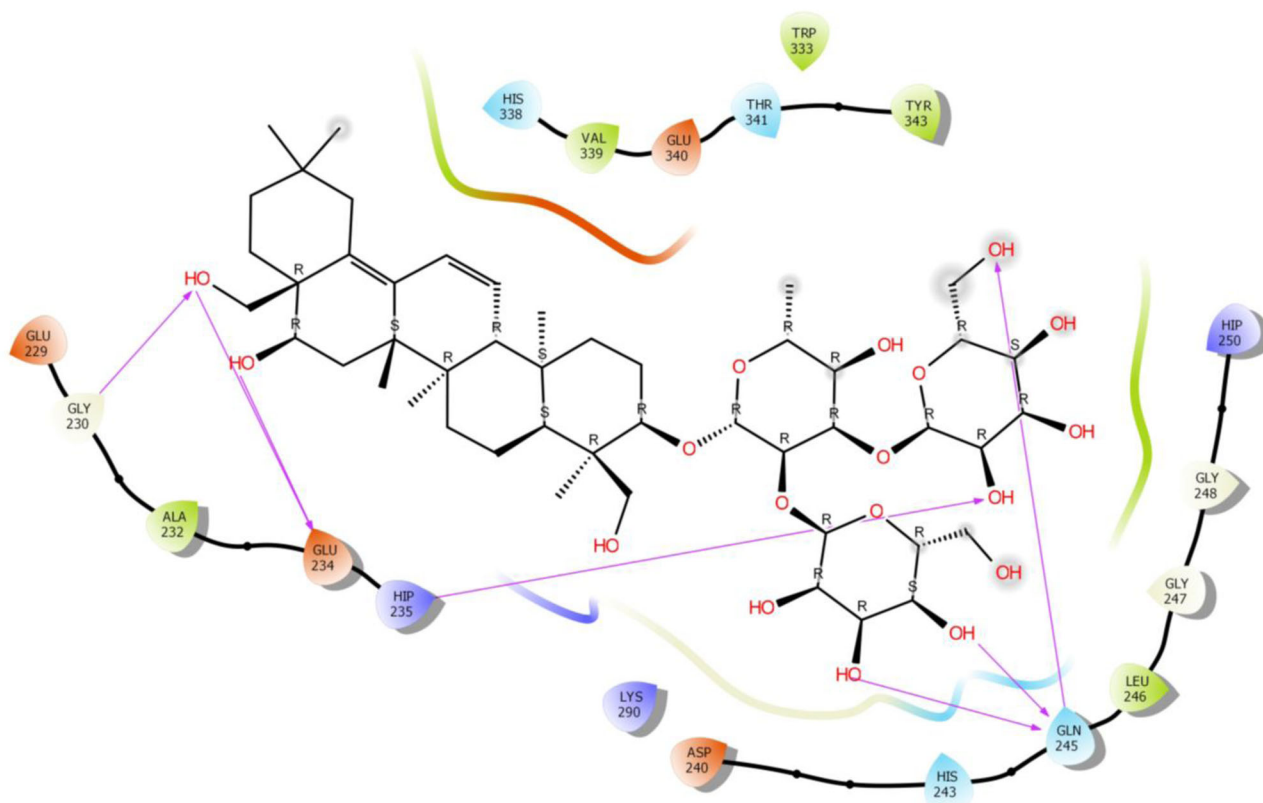


Figure 5. Binding-interaction analysis of Saikosaponin 1 b with NSP15 endoribonuclease (PDB ID: 6W01).

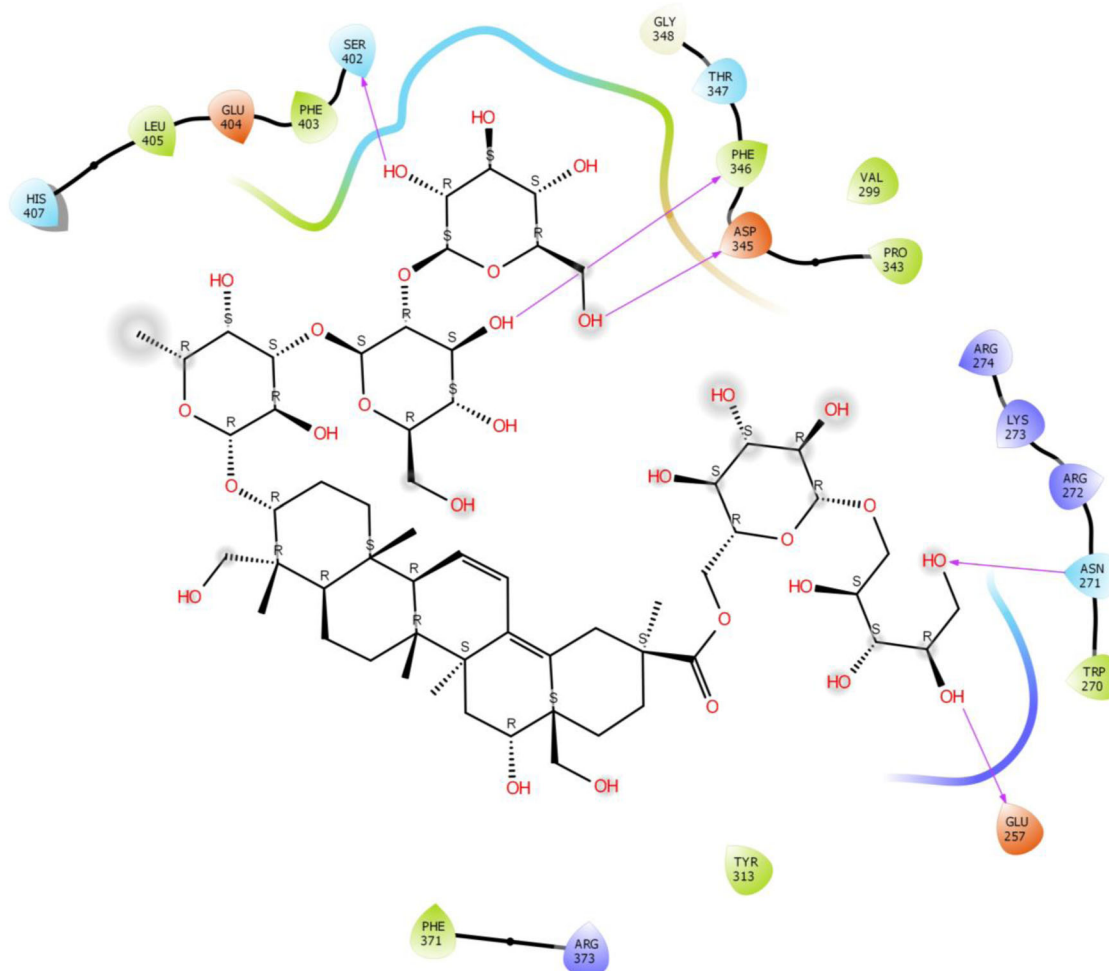
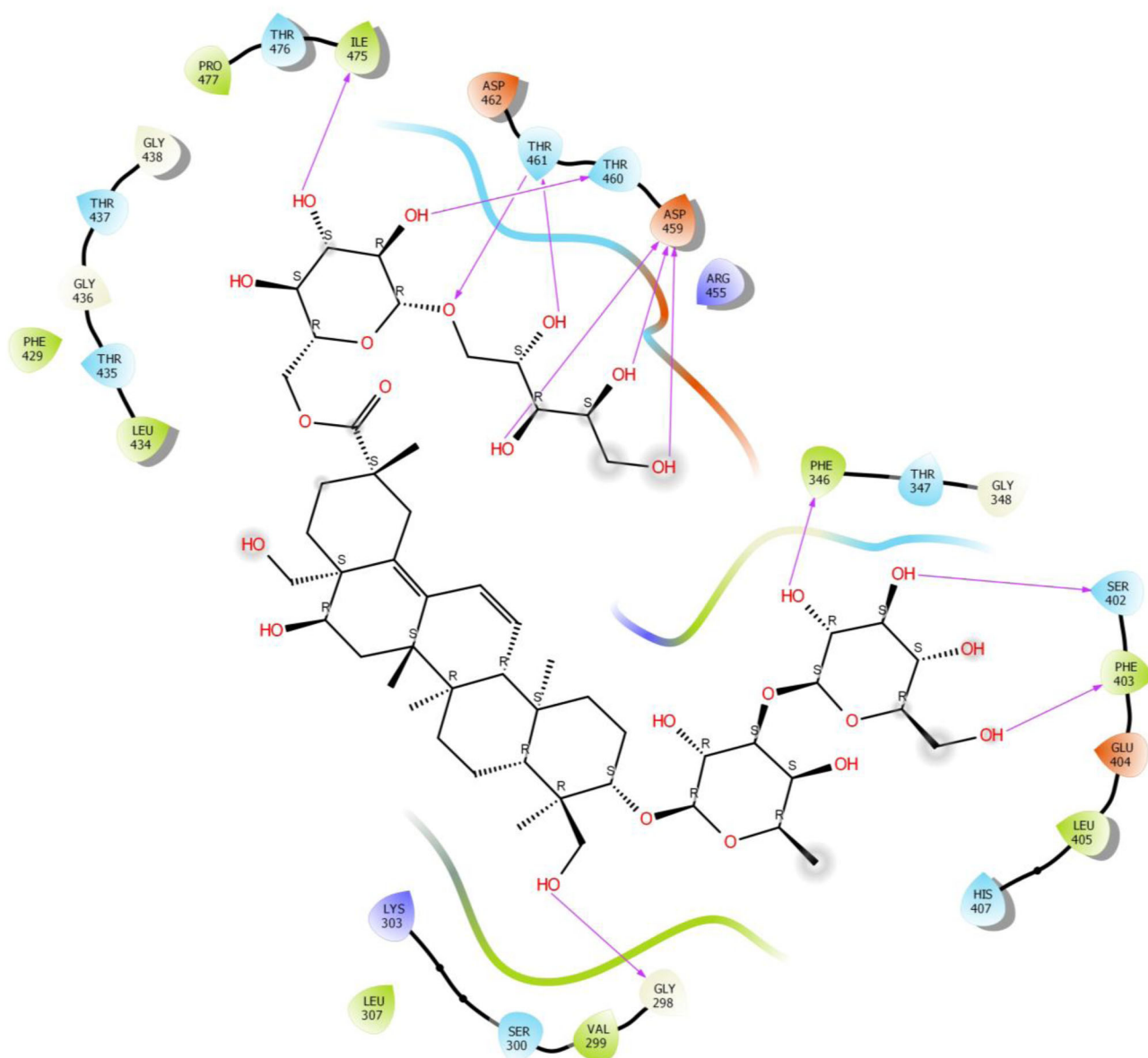


Figure 6. Binding-interaction analysis of Saikosaponin U with receptor-binding domain of spike glycoprotein (PDB ID: 6VSB).



**Figure 7.** Binding-interaction analysis of Saikosaponin V with receptor-binding domain of spike glycoprotein (PDB ID: 6VSB).

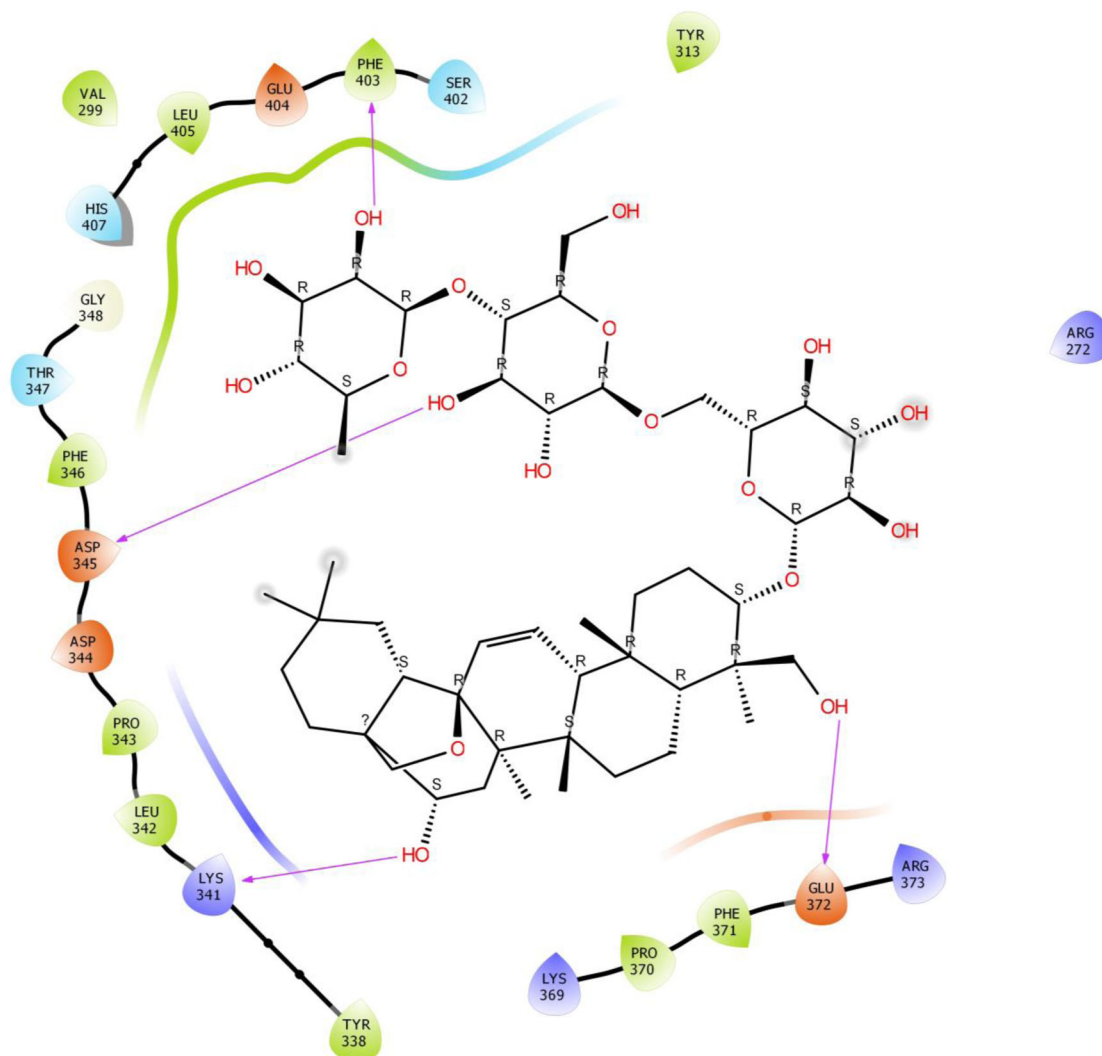
(NSP). Among these NSPs, the NSP15 is a nidoviral RNA uridylylate-specific Endoribonuclease (NendoU) which carries a catalytic C-terminal domain, which is specific for uridine (U). A recent study has reported that the NSP15 NendoU activity is mainly responsible for the interference of protein replication and innate immune response (Deng et al., 2017) and therefore, NSP15 is said to be very essential for biological progression of coronavirus.

The studies conducted on the structures of SARS- and MERS-CoV NSP15 revealed that the virus protein constitutes of hexamers i.e. dimers of trimers. Further, each monomeric unit is comprised of ~345 amino acids which are folded into three domains viz. N-terminal, middle and NendoU C-terminal catalytic domain, where the NendoU C-terminal catalytic domain comprises two  $\beta$ -sheets which are antiparallel. Study have reported that the NSP15 is responsible for cutting the double-stranded (ds) RNA substrates with specificity

via the  $Mn^{+2}$ -dependent Endoribonuclease activity. The main active sites, conserved among SARS-CoV-2, SARS-CoV and MERS-CoV proteins are located in a shallow groove between the two  $\beta$ -sheets that contain six key amino acids viz. His235, His250, Lys290, Thr341, Tyr343, and Ser294. Among them, His235, His250, Lys290 constitute the catalytic triad, His235 serves as general acid, His250 acts a base, while Ser294 together with Tyr343 have been found to govern U specificity.

Our observations revealed that, the Saikosaponin V having two oxane rings substituted with 3 hydroxyl group and the side chain contains 4 hydroxyl and an ester linkage showed very high binding with active site having a furrow between the two  $\beta$ -sheets, carries amino acids Lys290, Thr341, Tyr343 and Ser29. Among these Lys290 have been proposed to comprise the catalytic triad and Ser294 with Tyr343 are believed to govern U specificity. Four hydroxyl group of side





**Figure 8.** Binding-interaction analysis of Saikosaponin C with receptor-binding domain of spike glycoprotein (PDB ID: 6VSB).

chain provides a proper grip between hydrophobic pockets. Saikosaponin U having 2 hydroxyl and hydroxymethyl group of oxane ring and other 2 hydroxyl group and an ester linkage in side chain showed very high binding with active site carries key residues Lys290, Thr341, Tyr343, and Ser294. An ester linkage of side chain provides a proper grip between hydrophobic pockets. Saikosaponin C having two oxane ring substituted with 4 hydroxyl groups and the other chain containing furan ring, showed very high binding with active site carries residues Lys290, Thr341, Tyr343, and Ser294. Hydroxyl group of oxane ring provides a proper grip between hydrophobic pockets. Saikosaponin K is having 3 hydroxyl and hydroxymethyl group of oxane ring and other hydroxymethyl group in other chain showed very high binding with active site carrier key residues Lys290, Thr341, Tyr343, and Ser294. Hydroxyl and hydroxymethyl group of oxane ring provides a proper grip between hydrophobic pockets. Saikosaponin 1b having two oxane ring substituted with 3 hydroxyl and hydroxymethyl group and the other chain contains hydroxyl and hydroxymethyl group showed very high binding with active site carries residues Lys290, Thr341 and Tyr343. Hydroxymethyl group of other ring provides a proper grip between hydrophobic pocket.

It has been reported that, 2019-nCoV uses a densely glycosylated, homotrimeric class I fusion spike protein to make entry into the host cells. This S protein occurs in a metastable prefusion conformation which undergoes structural rearrangement so that the viral membrane gets fused with the host cell membrane (Li, 2016; Bosch et al., 2003). This fusion process is accelerated by binding of S1 subunit to the host-cell receptor and by transition of the S2 subunit to a highly stable postfusion conformation (Walls et al., 2017). The receptor-binding domain (RBD) of S1 subunit undergoes hinge-like conformational movement to engage the host-cell receptor, which transiently hide/expose the determinants of receptor binding. Further, the S1/S2 subunits have been reported to possess arginine-arginine-alanine-arginine (RRAR) a protease cleavage site. Recent studies have also reported that, 2019-nCoV S and SARS-CoV S shares the same functional host cell receptor i.e. angiotensin-converting enzyme 2 (ACE2). In addition, the 2019-nCoV RBD-SD1 106 fragment (S residues from 319–591) have reported to have ACE2 binding site. The affinity with which ACE2 binds to 2019-nCoV S ectodomain is 10- to 20-fold higher than ACE2 binding to SARS-CoV S. Due to the higher binding affinity of 2019-nCoV S for human ACE2, the spread of 2019-nCoV became

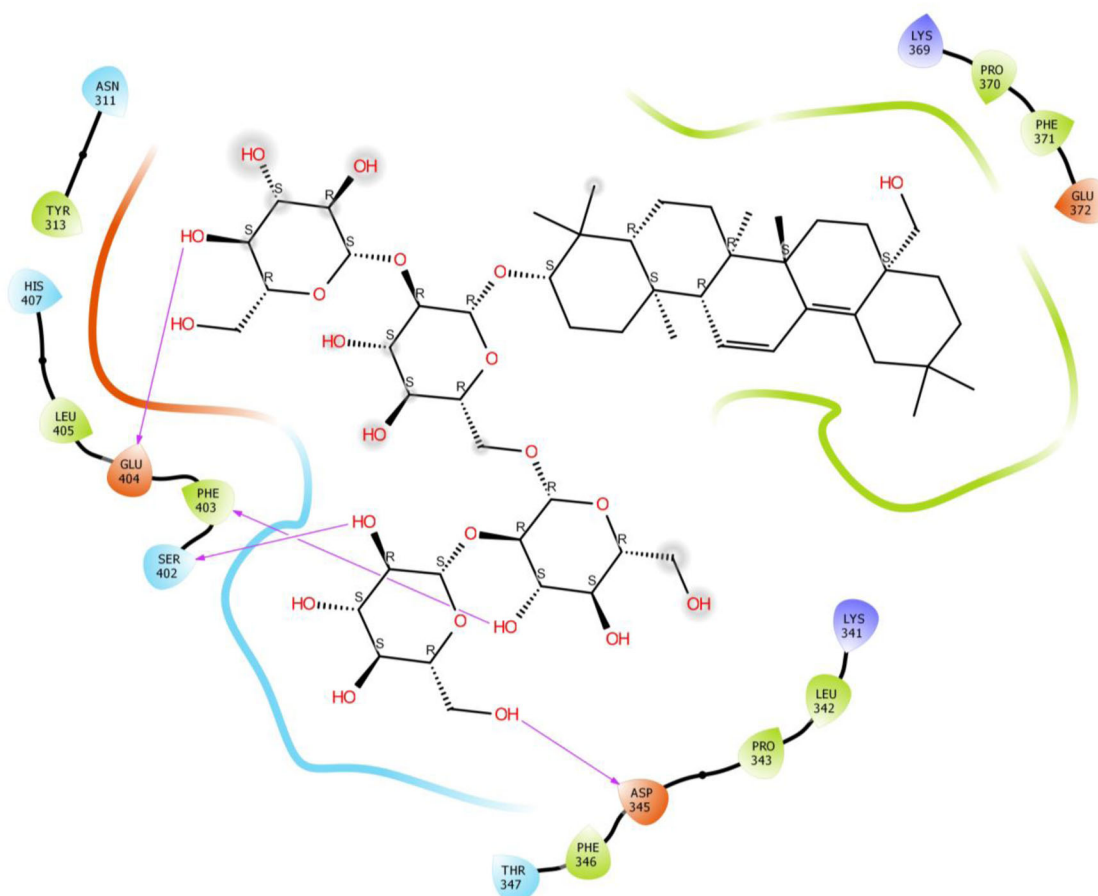


Figure 9. Binding-interaction analysis of Saikosaponin K with receptor-binding domain of spike glycoprotein (PDB ID: 6VSB).

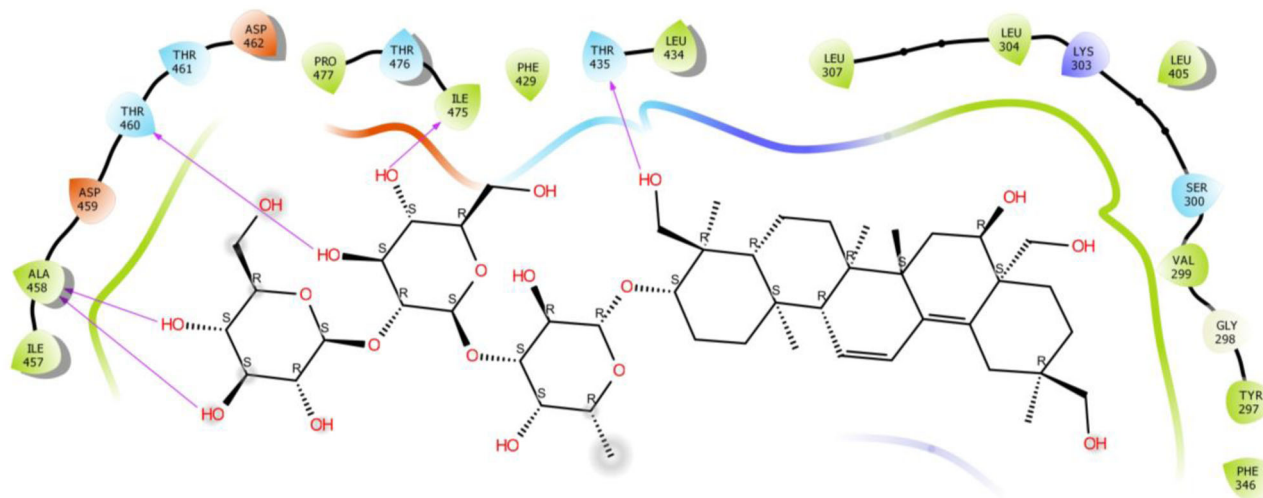


Figure 10. Binding-interaction analysis of Saikosaponin R with receptor-binding domain of spike glycoprotein (PDB ID: 6VSB).

apparently much prevalent from human-to-human (Chan et al., 2020).

Simulation study on crystal structure of 2019-nCoV spike glycoprotein revealed that all the Saikosaponin buried properly into the RBD of S1 subunit and also positioned into the N-terminal domain. Due to the help of RBD this virus makes connection with ACE 2 receptor and gets entry into the host cell. Based on the binding energy, Saikosaponin U and V were the top ranked ligand. The Saikosaponin U has

octadecahydropicene ring in which one side is substituted with 3 oxane ring and other side with one oxane ring. The Saikosaponin V having the same ring substituted with 2 oxane ring at one side whereas other side with one oxane rings. The extra oxane ring in Saikosaponin U provides better grip into the binding and hydrophobic pocket. The Saikosaponin U showed good interaction with triple arginine, which is responsible for recognition and cleavage of furin, this step facilitates the 2019-nCoV spike with ACE 2 receptor.

Saikosaponin R also having two oxane ring substituted with 4 hydroxyl and the side chain contains one hydroxymethyl group showed very high binding affinity and buried into the domain of binding site, out of which one hydroxyl group of oxane ring which provides a proper grip between hydrophobic pocket.

## Conclusion

In a nutshell, from all the simulation studies on both proteins, it can be concluded that Saikosaponin U and V have octadecahydronicene ring with substituted oxane ring but one more extra oxane ring of Saikosaponin U provide better grip into the wide spread binding pocket (residues from 319-519) of spike glycoprotein. The smaller structure of Saikosaponin V makes it best fit ligand into the narrow binding pocket of NSP 15. Thus we can say that Saikosaponin U and V would be the future research interest ligand as they mark the desire interaction with NSP15, which is responsible for replication of RNA and also with 2019-nCoV spike glycoprotein which manage the connection with ACE2.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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