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SHORT COMMUNICATION

Discovery of Immunomodulators from Plant Kingdom Targeting IL-6 for the Effective Management Therapy of SARS-CoV-2

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Abstract

The present study was conducted because of the recent scenario of this pandemic coronavirus outbreak worldwide. Currently, this disease cannot be treated through specific vaccines and therapeutic medicines. While many vaccines are being investigated, it would take some time for these to be accessible to the masses. Eventual evidence indicates that many COVID-19 patients may die from an irregular release of cytokines called as Cytokine Release Syndrome (CRS) due to the excessive reaction of their immune systems. In worsening patients with COVID-19, CRS played a significant role, from pneumonia via ARDS to cumulative systemic inflammation and eventually to a failing of the multi-system organ. In COVID-19 individuals, a large number of cytokines, including IL-6, IL-1, IL-2, IL-10, TNF- α, and IFN- α, participate in the 'cytokine storm,' but IL-6, whose higher serum levels are associated with respiratory failure, ARDS, and adverse clinical outcomes, tends to be a critical factor. In China, the COVID-19 mortality indicator has been tested by a multi-centre retrospective analysis in 150 COVID-19 patients. The study analysed that 82 cases are resolved from COVID-19 and 68 cases are dead due to enhancement of IL-6 levels in the serum. In this research, the secondary plant metabolites from Indian traditional medicine are identified through a computational technique and the selected seedling metabolite is sealed to block the IL-6 receptor.

Keywords: Covid-19, IL-6, Cytokine Release Syndrome, Secondary Metabolites

1. Introduction

According to emerging data, the Coronavirus has caused a new outbreak all over the world in the last year. It was observed for the first time in December 2019 in Wuhan, China. A coronavirus is a large group of family, which is a strain of the coronaviridae virus family. First, the infected animals transmitted this virus to humans (that means fats and pangolins), and then it spread throughout the world[1]. On February 11, 2020, the World Health Organization (WHO) designated this acute respiratory syndrome as coronavirus disease (COVID-19)[2]. The World Health Organization (WHO) in March 2020 recognized this disease as a global pandemic[3]. SARS-CoV-2 infected more than 212 countries with 5,529 195 cases worldwide 347,192 deaths from coronavirus statistics on 25 May 2020[2]. The COVID-19 transmission rate is reportedly very high, and an average of 3.8 individuals can be affected by each infected person. The death rates were also highly

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variable in COVID-19 patients and influenced by a range of variables like age and prevalence of underlying diseases[1]. The initial COVID-19 pathology revealed bilateral diffuse alveolar damage caused by cytomyxoid fibroma exudate, and a study of CD4+ and CD8+ T cells revealed a decrease in peripheral flow cytometry and an increase in Th17 cells. T_{17} cells are supported by mainly IL-6 (IL-6) and IL-23 activated and differentiated into Th_0 cells[4]. Recent instances of COVID 19 are associated with severe alveolar pneumonia leading to great acute breathing distress (ARDS) syndrome (up to 20 % of COVID 19 cases)[3]. Emerging evidence suggests that many COVID-19-infected patients may die from an abnormal release of cytokines known as Cytokine Release Syndrome (CRS), which is caused by an overreaction of their immune system. CRS influences the decline and deterioration of COVID-19 patients from pneumonia to Acute Respiratory Stress Syndrome (ARDS). CRS is a systemic inflammatory response characterised by increased pro-inflammatory cytokines due to infection, drug-drug interactions, and certain other external factors. It refers broadly to as an overactive immune response characterized by releases of interferons, interleukins, Tumor necrosis factor-alpha (TNF-α), chemokines, etc.[5] CRS is a prevalent immunopathogenesis that underlies much pathogenesis, including ARDS, sepsis, Graft-versus-Host Disease (GvHD), rheumatic disease-induced Macrophage Activation Syndrome (MAS), and primary and secondary Hemophagocytic Lymphohistiocytosis (HLH)[6]. Different infectious or non-infectious diseases can cause cytokine storms and severely damage multiple organs[7]. In many COVID-19 patients, including IL-6, IL-1, IL-2, IL-10, TNF-α, and IFN-β, many cytokines are involved in the 'cytokine storm;' however, IL-6 seems to play a crucial role, the increased levels of which have been associated with breathlessness, ARDS and adverse clinical outputs[2,8]. Surprisingly, critically ill patients had a sharply increased IL-6 level that was nearly 10 times that of severe patients, and all deaths had extremely high IL-6 values[9]. In addition to its role as an inflammatory mediator in innate and adaptive immune responses, the IL-6 cytokine can provide protection and anti-inflammatory properties in some pathological states. In addition, IL-6 and its receptors might have a high potential for diagnosis and treatment of the results of recent studies for covid-19 patients[1].

2. IL-6's Role in SARS-CoV-2

Weissenbach discovered IL-6 in 1980. In the cytokine network, IL-6 plays a significant role in acute inflammation. The human metabolism, Autoimmune Cell Differentiation, Disease Therapy, and so on. IL-6 is a cytokine with multiple functions. The IL-6 gene, which has four introns and five exons, is located on chromosome 7p15–21. Small, phosphorylated glycoproteins make up a large number of four-helix bundles, A, B, C, and D. Helixes A and B are moving in one direction, while Helixes C and D are moving in the opposite direction[1,4]. Several inflammatory cytokines, such as IL-1, IL-10, and Tumor Necrosis Factor (TNF- α), are about 2-100 times higher than normal in COVID-CSS, while IL-6 is significantly higher, in some cases over 1000 times higher[10]. Even moderately elevated IL-6 levels above 80 pg/mL were found to be sufficient for identifying COVID-19 infected patients at high risk of respiratory failure[11]. Monocytes, macrophages, endothelial cells, B and T cells, hepatocytes, keratinocytes, adipocytes, and fibroblasts are among the immune and stream cells that secrete IL-6. IL-6 is also assisted by a diverse set of functional IL-6 (IL-6R) receptors found in cells, B cells, endothelial vascular cells, monocytes, and hepatocytes[8]. Innate and adaptive immunity show the biological effects of IL-6. Concerning innate immunity, IL-6 activates an Anti-Microbial Peptide (AMP), APP, and C-reactive protein production in the liver via the JAK kinase pathway, a colony-stimulating factor that increases the monocyte differentiation into a macrophage, STAT-3 signalling pathway which modulates the maturation of dendritic cells. Concerning adaptive immunity, IL-6 enhances IgG, IL-4 production, distinguishes native T CD4+ lymphocytes. IL-6 blocks the Th1 (T-helper cell) which in turn leads to Th2 activation. As a result, in sick people with virus infections, elevated IL-6 tiers were associated with increased viral replication[1].

3. Research Methodology

3.1 Devices and Materials

In the molecular scenario in the modern drug design, docking is commonly used to understand the interaction between the target ligand-receptor and the target lead

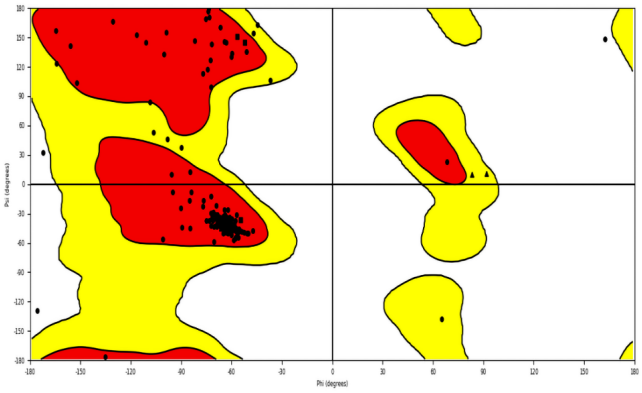


Figure 1. Ramachandran plot of 1ALU.

molecule's binding orientation with its protein receptor and is quite frequently used to detect the associations between the target components. The research work was done *in-silico* by utilizing bioinformatics tools. Also, by utilizing some of the offline programming's like Protein Data Bank (PDB), PubChem database, chem sketch. The ADMET and molecular docking studies were carried out through discovery studios (DS) 4.1[12].

3.2 Preparation of Protein

By utilizing the offline program Protein Data Bank (PDB), based on the literature survey, the human interleukin-6 (PDB: 1ALU) with a resolution of 1.90A^{o} was obtained. Then, the crystal water was deleted from the protein (1ALU) and added lacking hydrogens, protonation, ionization, and energy minimization. The CHARM (Chemistry of Harvard Molecular Mechanics) force field is applied for energy minimization[13]. Prepared protein is validated by utilizing the Ramachandran plot (Figure 1).

3.3 Preparation of Ligands

From the pieces of literature, take a library consisting of 152 secondary plant metabolites from the Indian traditional system. All 152 molecules are selected from the different literature works based on the particular compounds' immune modulation activity. Polysaccharides, terpenoids, flavonoids, alkaloids, glycosides, and lactones are important phytochemicals that have been linked to plant immunomodulation activity. These phytochemicals may serve as lead molecules in the development of safe and effective immunomodulators as potential treatments for viral disease prevention and cure. In addition, natural products have been shown to modulate the immune system in nonspecific ways. A number of plant-based principles with potential immunomodulation activity have been isolated and characterized, justifying their use in traditional folklore medicine and serving as the foundation for further research[14–17]. Herbal medicines are increasingly being used as multi-component agents to modulate the complex immune system to prevent infections rather than treat immune-related diseases. Many therapeutic effects of plant extracts have been proposed to be due to their diverse immunomodulatory effects and influence on the human body's immune system[18]. The use of phytomedicines has grown significantly over the last few decades. "Phytomedicines" and natural immunomodulators provide a safer alternative for prevention and treatment[19]. Through their effects on various cells via interleukins and cytokines, medicinal plants play an important role in the treatment of infections, inflammation, and immunodeficiencies[20]. Secondary plant metabolites from Indian traditional medicine are identified using a computational technique in this study, and the selected seedling metabolite is sealed to block the IL-6 receptor[21]. The molecules are designed in two and three-dimensional structures by utilising the Chem Draw Pro tool. After designing molecules, the structures were optimized in 3D optimization in chem draw and saved as an SDF format[22].

3.4 Docking Analysis

Discovery studios (DS) 4.1 was utilized to perform the docking analysis. Libdock and CDOCKER modules were used in discovery studios for virtual screening and binding affinity between the ligand and protein[23]. The workflow of our study is depicted in (Figure 2). Following the incorporation of both ligands and protein into the working environment. Libdock, a rigid-based docking program, was used to screen all 152 secondary plant metabolites for IL-6 (PDB: 1ALU). Libdock aids in the calculation of protein hotspots by employing polar and non-polar probes. Furthermore, based on a score known as the Libdock score, energy minimization ligands align into favourable interactions. The CHARM force field is used to perform the minimization in

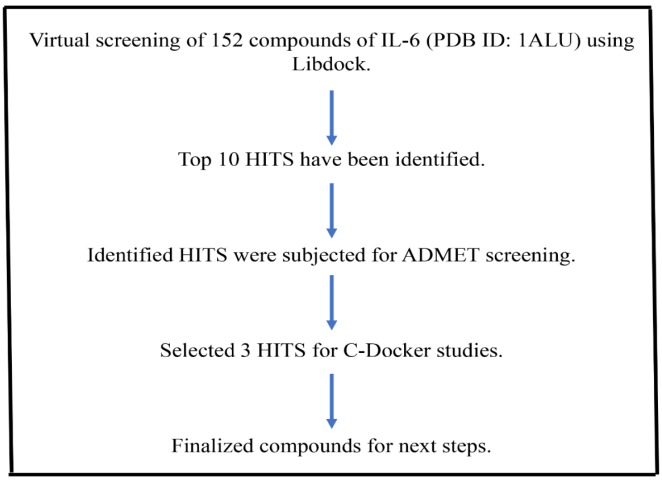


Figure 2. Workflow of our study.

2000 steps with an RMS gradient of 0.1. After the minimization, the option receptor-ligand interaction on the toolbar, then, need to select the 'Edit binding site' to define the binding site of the prepared protein. The active site of the docking was generated by using bound ligand binding positions. The virtual screening is carried out on Libdock, all prepared ligands on the defined active site. All the ligands are ranked according to the Libdock score[24].

3.5 ADME and Toxicity Prediction

To calculate the ADMET properties by utilizing the Discovery Studios (DS) 4.1, the ADMET are the modules of the discovery studios for predicting the ADMET properties. ADMET properties should be done for 10 hits which are showing the highest Libdock score. After the LibDock score followed by conducting ADMET studies on the top ten hits to determine their aqueous solubility, Blood-Brain Barrier (BBB) penetration, Plasma Protein Binding (PPB) level, hepatotoxicity, polar surface area, cytochrome P450, CYP2D6 inhibition, human intestinal absorption, rodent carcinogenicity, Ames mutagenicity, and developmental toxicology potential. A logP value, represents the partition coefficient values of molecules in the octanol/water system and indicates the lipophilicity of the molecules. LogP value is an important parameter that shows the effect on bioavailability, distribution, clearance of volume, membrane permeability[25].

3.6 Toxicity Prediction

In this study, the predictions and substantial descriptors of the molecules, such as mutagenicity and toxicological levels, were evaluated for various tissues. The PreADMET serve has predicted pharmacologically relevant properties (http://preadmet.bmdrc.org/).

3.7 Molecular Docking

CDOCKER is a module of Discovery Studios (DS) 4.1 version. CDOCKER is mainly used for the protein-ligand binding energies and binding interaction of the protein. It was done based on the CHARM docking tool. During docking, CDOCKER makes the protein as rigid and ligands are flexible. Each pose of the ligand gives the binding energies and interaction energy between the protein and ligand is calculated. All the isomers and tautomers of the ligands are usually known as poses. The lowest energy of each pose has been approved for the docking. In rigid and semi-flexible docking, water molecules are typically deleted before hydrogen atoms are added to the protein. The CHARM force field is used for both receptor and ligands[26].

4. Results and Discussion

The inflammatory response activates the immune system when a pathogen contains the host cell, causing the pathogens to be removed and the tissue to be repaired. The highly pathogenic virus-like CoVs, on the other hand, cause a prolonged cytokine response, which can lead to the mortality rate in Covid patients. Due to excess cytokine reaction to CoV infection, viral sepsis continues, and uncontrolled systemic inflammation develops in the viral cell, which can lead to pneumonia, acute respiratory symptoms, respiratory and organ failure, shock, and death[27]. Many cytokines participate in the cytokine storm in COVID-19 patients, including IL-6, IL-1, IL-2, IL-10, TNF- α, and IFN- γ, but IL-6, whose increased serum levels have been linked to respiratory failure, ARDS, and poor patient outcomes, appears to play a critical role. Virtual screening was performed with the software Libdock for pandemic management and identification of new compounds that may inhibit the ligand-binding IL-6 protein (PDB ID: 1ALU). Discovery Studio Modules 3.5 (DS3.5, Accelrys, Inc., San Diego, CA, USA). The

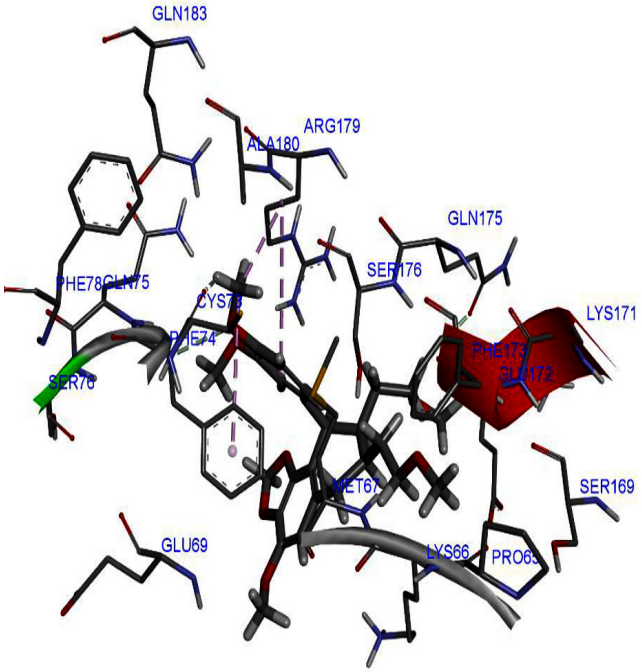


Figure 3. Amino acids present in the active site of the catalytic pocket of the IL-6 receptor (PDB id: 1ALU)

virtual screening for all the 152 compounds to the protein IL-6 (PDB id: 1ALU) was carried out.

4.1 Preparation of Protein and Identification of Active Site

Figure 3 depicts the 3D structure of the human protein interleukin-6 obtained from the protein data bank (PDB: 1ALU). The amino acid residues present in a catalytic pocket are *Gln183, Phe78, Gln75, Ser76, Ala180, Arg179, Cys78, Glu69, Ser176, Met67, Gln175, Phe173, Lys171, Lys66, Pro65, Ser169, Glu172* (Figure 3).

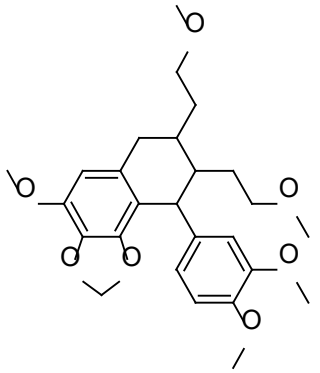
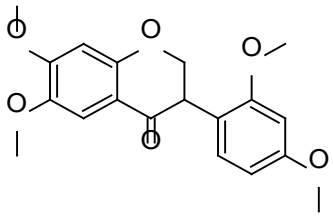
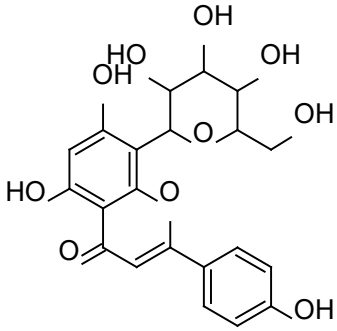
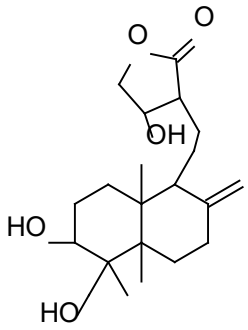
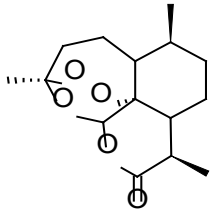
4.2 Libdock Score

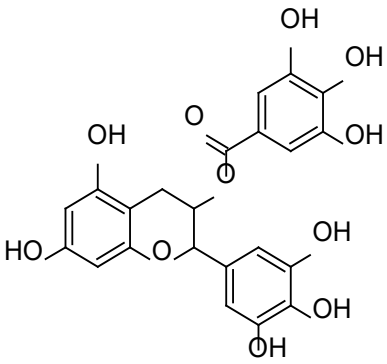
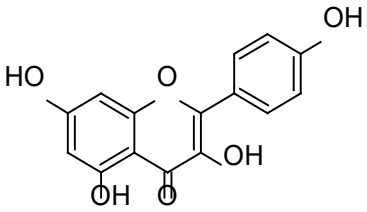
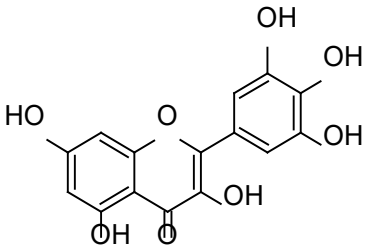
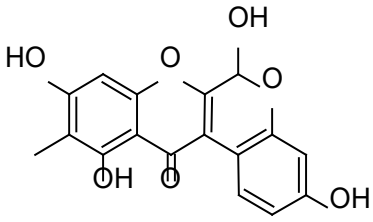
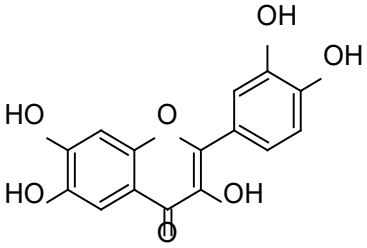
Virtual screening was performed with the software Libdock for pandemic management and identification of new compounds that may inhibit the ligand-binding IL-6 protein (PDB ID: 1ALU). Discovery Studio Modules 3.5 (DS3.5, Accelrys, Inc., San Diego, CA, USA). The virtual screening for all the 152 compounds to the protein IL-6 (PDB id: 1ALU). The standard was Glycyrrhizin (Table 1) is attached to the Libdock score. The score was based on the top ten compounds chosen for additional study, for instance, Arjunone, hypophyllanthin, vitexin, andrographolide, artemisinin, epigallocatechin, kaempferol, myricetin punarnava A, quercetin. The compounds were higher in Libdock. Libdock is a rigid docking method that calculates the protein's hotspots for all ligands and their interactions.

4.3 ADME Results

For ADME analysis, the top ten Libdock scorers were selected, and three compound compounds, namely Arjunone, Hypophyllanthin, vitexin, had good ADMET properties and druggable features. For the performance of the ADMET studies, the Discovery studio included the ADMET protocol. Compound lipophilicity and the AlogP value are used to represent a partition coefficient of values (logarithm) of octanol and water compounds. It is a critical property that influences bioavailability, membrane permeability, and drug distribution and clearance routes. This parameter is critical in predicting pharmacodynamics and toxicology. All three compounds showed logP values less than five, which showed the limits of these compounds. The effective concentration of medicinal products depends not only on absorption and metabolism but also on the binding of plasma protein (PPB). The binding of plasma protein affects drug distribution and can be classified as binding restrictive and permissive. Therefore, the efficiency of a medication is also measured. ADMET also identifies the inverse correlation between the Polar Surface Area (PSA) and human intestinal absorption. The trust ellipse of 99% in the area of chemical space compounds with outstanding cell membrane absorption. The PSA < 140 Å2 and AlogP98 < 5 criteria were shown to be a compound with ideal cell permeability. PSA< 140 Å2 showed all of the compounds are within the limit shown in (Figure 4). All compounds had high absorption and were polar. The levels of blood barrier were high, respectively, for all the compounds were shown in (Table 2).

Table 1. Top 10 hits of Libdock score

S.no	Ligand	Structure	Libdock score
1	Hypophyllanthin		184.88
2	Arjunone		179.72
3	Vitexin		175.51
4	Andrographolide		173.33
5	Artemisinin		172.76

6	Epigallocatechin		172.23
7	kaempferol		167.03
8	Myricetin		165.38
9	Punarnava A		165.32
10	Quercetin		164.68

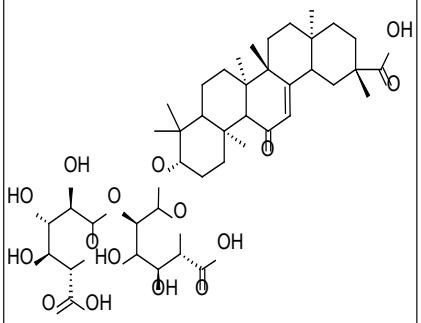
11	Glycyrrhizin		177.63

Table 2. ADMET properties of selected compounds

Compound	solubility[a]	BBB[b]	CYP2D6[c]	hepatotoxicity[d]	PPB[e]	absorption[f]	Alogp9[g]	PSA[h]
Arjunone	2	1	False	True	True	0	4.127	62.51
Hypophyllanthin	2	1	False	True	true	0	2.916	61.95
Vitexin	2	1	False	True	True	0	1.728	59.49

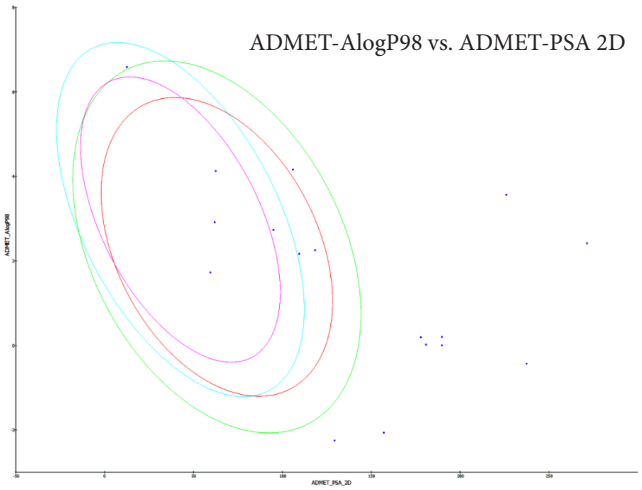


Figure 4. Plot of PSA versus AlogP with 95% and 99% confidence limit ellipses.

[a]Solubility level- 0 extremely low, 1 no very low but possible, 2 yes low, 3 yes good, 4 yes optimal, 5 no too soluble; [b]BBB- Blood-brain barrier level-0 very high, 1 high, 2 medium, 3 low, 4 undefined; [c]CYP2D6- Cytochrome 450 inhibition; [d] Hepatotoxicity; [e]PPB- Plasma-protein binding; [f] Absorption- 1 very poor, 2 Poor, 3 Medium, 4 Good; [g]AlogP98- Partition coefficient of octanol /water system; [h] PSA- Polar surface area

A human cytochrome enzyme P450 is a superfamily gene member that is versatile in that it can easily oxidize hydrophobic drugs and eliminate foreign matter. Among this main family of CYPs, the three isoforms 2D6, 2C9, and 3A4, considered microsomal oxidation of most human drugs to be more important and responsible. The metabolism of CYP2D6 exceeds 27.5% of drugs and largely considers the bioconversion of many xenobiotics to be polymorphic metabolizing enzymes. As a result, we chose this isoform to predict the ADMET property of the compounds. For the CYP2D6 isoform, there are two types of ADMET for predicting activity: non-inhibitor (false) and inhibitor (true). Non-inhibitors of CYP2D6, a metabolic enzyme in the liver, have been predicted to be one of the most important CYP isoforms, and none of them are expected to cause significant drug interaction toxicity. The Bayesian score for all compounds was less than 0.162, indicating that predicted compounds were metabolized in the liver.

4.4 Toxicity Analysis

For the safest and most effective medicines, the pharmaceutical industry has a huge challenge. PreADMET online software assessed the prediction

for toxicity of the top 3 compounds. According to the results of (Table 3), Arjunone, hypophyllanthin, and vitexin do not show carcinogenicity in mouse and rats, but arjunone show carcinogenicity in rats. The PreADMET analysis has enabled compounds to check whether hERG is inhibited. The composition contains a high risk of inhibiting hERG, which covers the potassium channel participating in heart repolarization. Inhibiting hERG may result in an extension of the QT ranges, which may result in a potentially fatal ventricular tachyarrhythmia known as Torsade de Pointes (electrocardiographic parameter representing the duration of electric systole – cardiac contraction). In this case, Arjunone shows medium risk, hypophyllanthin shows low risk, and finally, vitexin show high risk. Therefore, vitexin is cautious in the case of cardiopathies. The Ames test is a simple method for determining a compound's mutagenic potential against the *Salmonella typhimurium* strain. Using PreADMET, the mutagenic activity of TA100_NA and TA153 _10RLI is found to be positive. The mutagenic activity was found to be negative for TA100_10RLI and TA1535_NA. Ames testing does not necessarily require mutagens to be carcinogenic. These tests are being used to analysis showed the toxicity of chemical compounds. From these results, all our compounds are low toxicity and carcinogenicity.

4.5 Molecular Docking

The CDOCKER interaction energy parameters were considered in selecting the best "HITS" and compared with the known inhibitor glycyrrhizin. CDOCKER interaction energy is the energy for the interaction between the protein and the ligand. This strongly indicates the value of the two parameters for the interaction of proteins and ligands. The interaction energy of the CDOCKER components was above standard glycyrrhizin (26.3636) shown in (Table 4), indicating that the interaction energy of the CDOCKER three compounds could be more bonded by IL-6. The hydrogen bonds and Pi-Pi interactions of IL-6 and these compounds were analysed as shown in (Figure 5). In Arjunone and IL-6 protein domain ligand complex made three carbon-hydrogen bonding (Met67, Gln175, Cys73), eight hydrophobic interactions (Lys171, Pro65, Phe173, Ser169, Lys66, Ala180, Gln183, Gln75), one pi-interaction (Arg179), and one pi-alkyl (Phe74). Hypophyllanthin and IL-6 protein domain complex made one conventional hydrogen bonding (Lys69), five hydrophobic interactions (Met67, Ala68, Gln183, Ser176, Ala180), pi-interaction (Arg179). Vitexin and IL-6 protein domain complex made three hydrogen bonds (Arg179, Arg33, Gln175), four hydrophobic interactions (Arg40, Ser37, Ile36, Lys171), pi-interaction (Arg30, Leu33).

Table 3. Toxicity predictions by PreADMET software

Descriptors	Arjunone	Hypophyllanthin	Vitexin
algae_at	0.0414292	0.0324254	0.0137471
Ames_test	mutagen	mutagen	mutagen
Carcino_Mouse	negative	negative	negative
Carcino_Rat	positive	negative	negative
daphnia_at	0.0933798	0.0628534	0.219746
hERG_inhibition	medium risk	low risk	high-risk
medaka_at	0.0146563	0.00733921	0.093154
minnow_at	0.0160126	0.012854	0.124993
TA100_10RLI	positive	negative	negative
TA100_NA	positive	negative	negative
TA1535_10RLI	negative	negative	negative
TA1535_NA	negative	negative	negative

Table 4. Binding interaction energies of top compounds

S.no	Compound	CDOCKER Interaction Energy (-Kcal/mol)
1	Arjunone	38.585
2	Hypophyllanthin	30.570
3	Vitexin	26.8662
4	Standard compound	26.3636

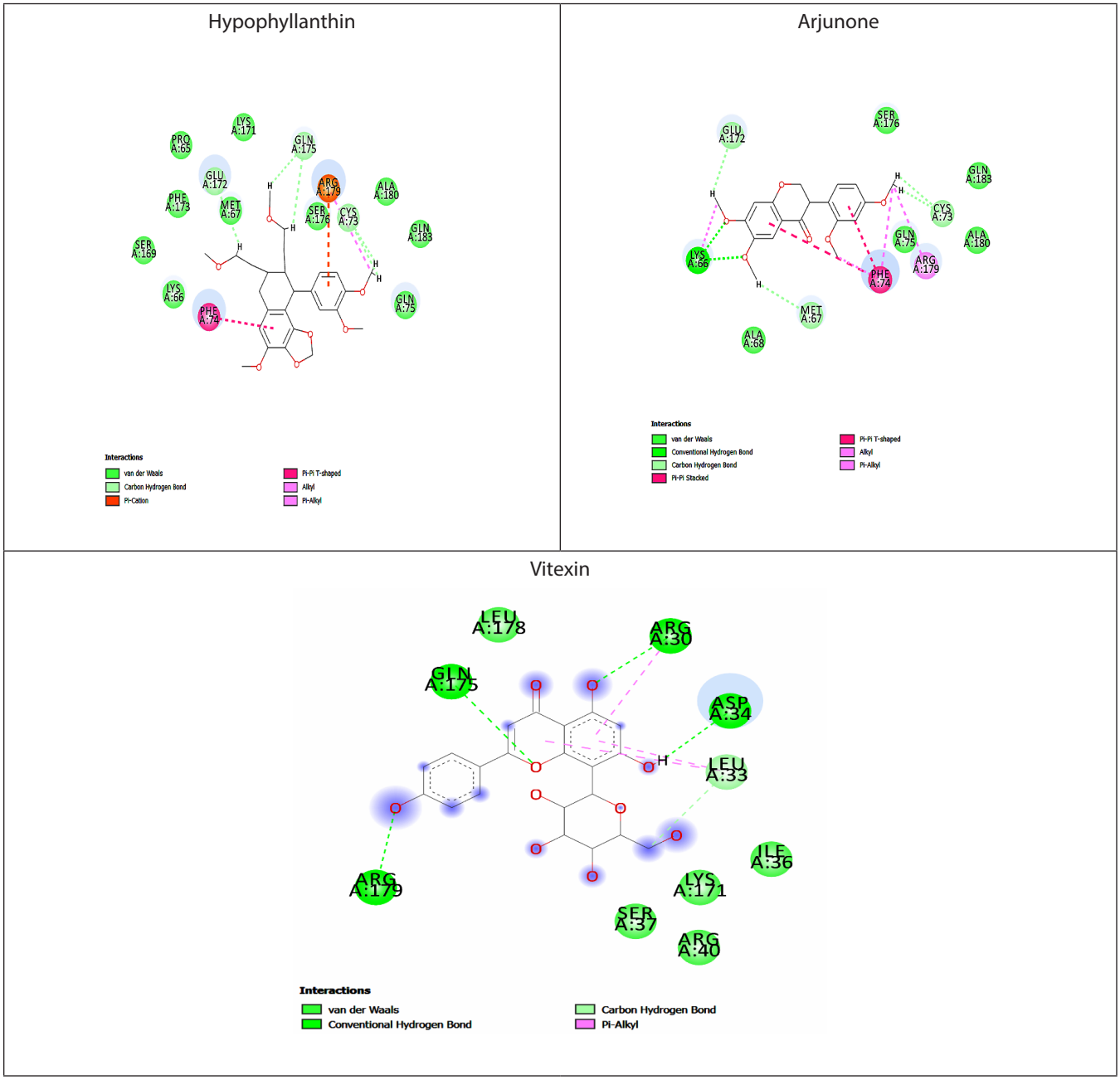


Figure 5. 2D Interactions of the compounds.

5. Conclusion

There is an immediate need to respond to the outbreak accelerate research to discover treatment and cure for the latest coronavirus disease through a herbal source. The current research has a wide range, as so far, no such strong and cost-effective medicine has been developed from traditional Indian medicine for SARS-CoV care. In this present research work, IL-6 plays a very predominant role in SARS-CoV due to a large amount of cytokine release leading to inflammation in the lungs and finally leading to death. The IL-6 levels are associated in this setting with respiratory failure, bad results, and mortality. The early suppression of IL-6 and signals promises novel and unnecessary immune functions for the development of acute SARS-CoV-2 infection. So, based on the literature survey, we have selected some plant-derived immunomodulators for IL-6 inhibition. By utilizing discovery studios software, done the docking studies; in that, we find the top 3 molecules are best suitable for IL-6 inhibition. The top 3 molecules are revealed through in-silico docking studies are arjunone, hypophyllanthin, and vitexin analogs, which showed an almost nearby docking score with IL-6. Furthermore, patients receiving Indian integrated medicine believed that they played a significant role in the diagnosis of Covid-19 in the early stages of infection.

6. Conflict of Interest

The authors declare no conflicts of interest.

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