Phytocompounds of *Rheum emodi*, *Thymus serpyllum* and *Artemisia annua* inhibit COVID-19 binding to ACE2 receptor: *In silico* approach

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Research Article

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Abstract

COVID-19 has been declared as global epidemic and currently there is no drug/vaccine available to treat COVID-19. All over the world, several studies are being conducted to discover the antiviral drugs against COVI-19. Traditional medicinal plants have long history to treat viral infections. We adopted *in silico* approach to find out if unique phytocompounds such as emodin (*Rheum emodi)*, thymol and carvacrol (*Thymus serpyllum)* and artemisnin (*Artemisia annua)* could physically bind COVID-19 target proteins such as SARS-CoV-2 spike glycoprotein (PDB ID: 6VXX), SARS-CoV-2 spike ectodomain structure (PDB ID: 6VYB), and SARS coronavirus spike receptor-binding domain (PDB ID: 2AJF) and in turn preventCOVID-19 binding to the host receptor ACE2. Since Chloroquine (a standard antimalarial drug) has been looked as potential therapy against COVID-19, we also compared the binding of chloroquine and plant origin artemisnin antimalarial drug for its interaction with 6VXX, 6VY and 2AJF. Molecular docking studies using AutoDock/Vina software revealed that among all the phytocompounds artemisinin showed best binding affinity with 6VXX, 6VYB and 2AJF with E_{total} -10.5 KJ mol^{-1}, -10.3 KJ mol^{-1}, and -9.1 KJ mol^{-1} respectively. Whereas emodin, carvacrol and thymol binds with 6VXX, 6VYB and 2AJF with E_{total} -6.4, -6.8, -6.9 KJ mol^{-1}, -8.8, -6.8, -7.4 KJ mol^{-1}, and -6.9, -7.4, -7.2 respectively. Similarly, with Autodock/Vina chloroquine showed less binding affinity with 6VXX (-5.6 KJ mol^{-1}), 6VYB (-5.9 KJ mol^{-1}) and 2AJF (-6.4 KJ mol^{-1}) as compared to all phytocompounds. Toxicity prediction showed non-toxicity and non-carcinogen by admetSAR and PROTOX-II software.

Introduction

COVID-19 caused by a family of Coronavirus (CoV) has threatened the survival of Human beings on the Earth and it has been declared as global health emergency by World Health Organization (WHO) (Sohrabi *et al*., 2020). Coronavirus (CoV) comes under the family of viruses which has the ability to cause illness. It starts with the common cold and ends with more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Several Acute Respiratory Syndrome (SARS-CoV). A new strain novel corona virus (nCoV) has been come into knowledge which is mainly found in humans. In Latin, Corona means “halo” or “crown” thus the name represents the structure of the virus which consists of crown like projections on its surface (Islam, 2017). In 1937, corona virus was isolated from an infectious bronchitis virus in birds which was responsible to ruin the poultry stocks (Bracewell, 1977). Further, human corona virus was identified in the year 1960 in the nose of patient suffering from common cold and OC43 and 229E were the two human corona viruses responsible for common cold. Corona viruses are the type of viruses that directly affect the respiratory tract. These are associated with the common cold, pneumonia, gut as well as severe acute respiratory syndrome. Corona viruses are zoonotic, which means they are transmitted between animals and humans (Ksiazek *et al*., 2003).

The first case of Middle East respiratory syndrome (MERS-CoV) was seen in the year 2012, a businessman in Saudi Arabia who died from viral pneumonia (Zaki *et al*., 2012). Later, this disease

started appearing in the people living outside the Saudi Arabia due to their close contact with individual living in other country (European Centre for Disease Prevention and Control, Severe respiratory disease associated with MERS-CoV, Stockholm: ECDC; 11 June 2015) in 2016, a report on 1698 was published by World Health Organization (WHO) regarding the confirmed cases of MERS-CoV infection and the death rate was approximately 36% (Middle East Respiratory Coronavirus (MERS-CoV) Bialek *et al*. (2014). The biggest outbreak with first ever confirmed case of this disease came into existence in the year 2015 in South Korea. Including the China, the confirmed cases extend to 186 with total 36 deaths (Cowling *et al*., 2015). Recently, case regarding the novel coronavirus came in to existence among the population of Wuhan, China on December 8, 2019. Pneumonia was the first symptom of infection and most of the cases were linked to a local fish and animal market. During the research it was seen that 2019 novel coronavirus was recognized as pathogenic agent responsible for evolution of pneumonia (Zhu *et al*., 2019). At the very first China was unaware about the direct transmission of 2019 novel coronavirus (2019-nCoV). However, patients without symptoms were recognized as the major source of infection as well as human-to-human transmission was also confirmed (Rothe *et al*., 2020). There was increase in the number of confirmed cases from 1 to 15. On January 20, 2020 laboratory in Korea confirmed the first case of Coronavirus. On 23 January, 2020, the government of China announced the total shutdown of country and advised the people for undergoing personal isolation. On 30 January, 2020, World Health Organization declared this problem as public health emergency of international concern (Sohrabi *et al*., 2020).

Virus particle consists mainly of four structural proteins. The spike (S), membrane (M), envelop (E), and proteins (N) are the four structural proteins which encodes with 3’ end of the viral genome (Wang *et al*., 2020; Zhou *et al*., 2018). Among all, S protein plays an important role in viral attachment, fusion, entry, and also act as a target for development of antibodies, entry inhibitors and vaccines (Du *et al*., 2009; Wang *et al*., 2016). The S1 domains of coronaviruses may contain receptor-binding domains (RBDs) that directly bind to the cellular receptors (Babcock *et al*., 2004; Wong *et al*., 2004). The SARS-CoV surface exhibit two components: S1, which contains the receptor binding domain (RBD); and S2, which contains the fusion peptide. SARS-CoV gains entry into cells through interaction of the SARS-SRBD with the cell surface receptor angiotensin converting enzyme 2 (ACE2) (Anand *et al*., 2003; Perlman *et al*., 2009). These interactions are followed by endocytosis, and at the low pH in endosomes, SARS-S is cleaved by a cellular protease called cathepsin L, thereby exposing the S2 domain of the spike protein for membrane fusion (Bosch *et al*., 2003; Yang *et al*., 2004). The minimal RBD of SARS-CoV S protein is located in the S1 subunit (AA 318–510) and is responsible for viral binding to host cell receptors (Dimitrov, 2003; Xiao *et al*., 2003). Besides the main receptor, angiotensin-converting enzyme 2, there are several alternative receptors, such as dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin and/or liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin (DeDiego *et al*., 2011). SARS-CoV and MERS-CoV RBDs recognize different receptors. SARS-CoV recognizes angiotensin-converting enzyme 2 (ACE2) as its receptor, whereas MERS-CoV recognizes dipeptidyl peptidase 4 (DPP4) as its receptor (Li *et al*., 2003; Raj *et al*., 2013). Similar to SARSCoV, SARS-CoV-2 also recognizes ACE2 as its host receptor binding to viral S protein (Zhou *et al*., 2020). Two residues (AA 479 and AA 487) in RBD

determine SARS progression and tropism, and their mutations may enhance animal-to-human or human-to-human transmission (Wong *et al*., 2004). Some residues (AA 109, 118, 119, 158, 227, 589 and 699) in S protein are critical strategies against this deadly viral agent, especially in high-risk groups, including people of every age group (Cinatl *et al*., 2003).According to the previous data, the ACE2 receptor expressing cell fused with SARS-S- expressing cells adds to the cell surface by pH independent mechanism (Xiao *et al*., 2003). It enhances the cell stress responses and apoptosis (Jeffers *et al*., 2004). Binding is very critical for pathogenesis if the binding is blocked, then COVID will not bind with Human cell receptor (ACE2), hence infection stopped. Traditional medicinal plants produce large number of compounds which is used as therapeutics to kill the pathogens (Iyengar, 1985). In the recent years so many reports published on antimicrobial activity of the medicinal plants. It is expected that plant extracts and phytocompounds showing the target site other than antibiotics, a very little information is available on this type of activity of medicinal plants (Hasegawa *et al*., 1995; Lee *et al*., 1998). Extracts of medicinal plants has been used from ancient times and these plants are known for their antiviral properties and less side effects. Traditionally, thyme was acclimated to treat asthma and loosen congestion in the throat and stomach (Heilmeyer, 2007). The pharmacological manuscript of Chailander medical codex (15th and 16th centuries) mentions the utilizations of wild thyme for the treatment of headaches caused by cold and laryngitis (Jaric *et al*., 2014). During the Renaissance period (16th and 17th centuries), wild thyme was utilized internally to treat malaria and epilepsy (Adams *et al*., 2012). Traditionally in many countries areal part of *T. serpyllum* utilized as anthelmintic, a vigorous antiseptic, an antispasmodic, a carminative, deodorant, diaphoretic, disinfectant, expectorant, sedative, and tonic (Chevallier, 1996). *Thymus serpyllum* additionally used to treat respiratory quandaries (Menkovic *et al*., 2011; Jarić *et al*., 2015). In western Balkans thymus species used to amend blood circulation and as anticholesterolemic, immunostimulant (Mustafa *et al*., 2015). Carvacrol and thymol are isomers, belonging to the group of monoterpenic phenols with potent antiseptic properties. Chauhan *et al*. (2010) reported thymol (25-200 mg kg^{-1}) as immunomodulatory in cyclosporine – A, treated Swiss albino mice by enhancing the expression of cluster of differentiation 4 (CD4), cluster of differentiation 8 (CD 8) and Th1 cytokines via upragulation of IFN-4expression and enhanced secreation of interleukin -12 (IL-12). Thymol increase the production of antibody titers against the Nwcastle diseases virus in broiler chickens (Khajeal *et al*., 2012).

Antiviral property of *Thymus serpyllum* (Herrmann *et al*., 1969) and thymol is already reported (Rustaiyan *et al*., 2000). Pilau *et al*. (2011) reported the antiviral activity of carvacrol from *Lippia graveolens* against human and animal virus (herpes simplex virus, acyclovir-resistant herpes simplex virus 1, bovine herpesvirus 2, respiratory syncytial virus; human rotavirus, bovine viral diarrhoea virus). Antiviral nature of Emodin was also reported in several studies (Hsiang and Ho, 2008; Dai *et al*., 2017; Zhu *et al.,* 2019). Study from Efferth *et al*. (2008) showed *in vitro* antiviral properties of artemisinin against Hepatitis B virus, Hepatitis C virus and Bovine viral diarrhea. Keeping in view the antiviral potential of Himalayan herbs, the current study was focused on the identification of potent phytocompounds from Himalayan herbs (*Rheum emodi*, *Thymus serpyllum* and *Artemisia annua*) to cure a dangerous COVID-19 (Fig. 1).

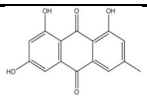
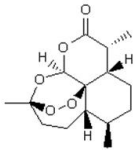
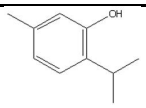
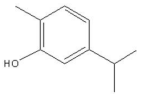
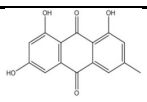
Methods

Bioinformatics tools: Open Babel GUI (O'Boyle *et al*., 2011), UCSF Chimera 1.8.1(Pettersen *et al.,* 2004), Pubchem (www.pubchem.com), RCSB PDB (http://www.rscb.org/pdb), PDBsum (www.ebi.ac.uk/pdbsum), and Autodock software (Trott and Olson, 2010) were used in the present investigation.

Ligand preparation

Four major phytocompounds of three medicinal plants- emodin of *Rheum emodi*, thymol, and carvacrol of *Thymus serpyllum* and artemisnin of *Artemisia annua* were used for the docking studies. The 3-dimensional structures of all the phytocompounds and chloroquine were obtained from pubchem (www.pubchem.com) in .sdf format. The .sdf file of the phytocompounds was converted into PDB and pdbqt format by using the Open Babel tool (Wang *et al.,* 2009). Table-1 showed molecular structure, molecular weight, pharmacological properties, plant source, percentage of phytocompounds in plant and antimalarial drug chloroquine. Tragets of phytocompounds and standard drug chloroquine was predicted by using SwissADME online server.

Table 1: Molecular structure, molecular weight, and pharmacological properties plant source and percentage of selected phytocompounds and chloroquine.

me of tocompounds	Plant source	Molecular structures	Percentage (%) of phytocompounds in plants	Molecular weight (g mol-1)	Pharmacological properties
odin	Rheum emodi		23.24 (Rolta et al., 2020)	270.24	Antiviral (Schwarz et al., 2011), antimicrobial (Cao et al., 2015)
emisinin	Artemesia annua		0.77–1.06 (Castilho et al., 2008)	282.33	Antimalarial (Tu youyou, 2011); Antiviral (Efferth et al., 2008)
ymol	Thymus serpyllum		8.3 (Gul et al., 2018)	150.22	Antiseptic, antibacterial, antifungal and antioxidant properties (Jeric et al., 2015), Antivirotic (Rustaiyan et al., 2000)
rvacrol	Thymus serpyllum		3.03 (Gul et al., 2018)	150.22	Antimicrobial, antithrombotic, anti-inflammatory, acetyl cholinesterase inhibitory properties (Jeric et al., 2015), Antiparasitic (Can Baser, 2008), Antiviral (Pilau et al., 2011)
loroquine	Standred antimalarial drug		Standard Antimalarial drug	319.9	FDA is allowing the use hydroxychloroquine treat coronavirus disease 2019 (COVID-19) (Jaffe, 2020).

Protein preparation

Three target proteins of COVID-19 were SARS-CoV-2 spike glycoprotein (PDB ID: 6VXX) and SARS-CoV-2 spike ectodomain structure (PDB ID: 6VYB) (Walls *et al.,* 2020) and SARS coronavirus spike receptor-binding domain, (PDB ID: 2AJF) (Li *et al*., 2005) were used to analyze the interactions of major phytocompounds of *R. emodi, T. serpyllum* and *A. annua.* The 3-Dimensional structures of selected target proteins were retrieved from PDB (Protein Data Bank) (http://www.rscb.org/pdb). Both proteins had co-crystallized ligand (X-ray ligand) in their binding site. The complexes bound to the receptor molecule, such as non-essential water molecules, including heteroatoms were removed from the target receptor molecule and hydrogen atoms were added to the target receptor molecule. Active site of three target proteins of COVID-19 were SARS-CoV-2 spike glycoprotein (PDB ID: 6VXX) and SARS-CoV-2 spike ectodomain structure (PDB ID: 6VYB) and SARS coronavirus spike receptor-binding domain was determined by grid box generation. Grid box was generated by adjusting the grid parameter (X, Y, Z coordinates values) using AutoDock software. The grid values were recorded in the config.txt file format. Three dimensional structures of target proteins of COVID-19 proteins are shown in Fig. 2.

ADMET and toxicity prediction of phytocompounds

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) screening was done to determine the absorption, toxicity, and drug-likeness properties of the ligands.The 3-Dimensional structures of ligands such as emodin, thymol carvacrol, artemisinin and chloroquine were saved in .smiles format and drug were uploaded on SWISSADME (Molecular Modeling Group of the SIB (Swiss Institute of Bioinformatics), Lausanne, Switzerland), admetSAR (Laboratory of Molecular Modeling and Design, Shanghai, China), and PROTOX webservers (Charite University of Medicine, Institute for Physiology, Structural Bioinformatics Group, Berlin, Germany) for ADMET screening. SWISSADME is a web tool used for the prediction of ADME and pharmacokinetic properties of a molecule. The predicted result consists of lipophilicity, water solubility, physicochemical properties, pharmacokinetics, drug-likeness, medicinal chemistry, and Brain or Intestinal Estimated permeation method (blood–brain barrier and PGP ± prediction) (Daina*et al.,* 2017). admetSAR provides ADMET profiles for query molecules and can predict about fifty ADMET properties. Toxicity classes are as follows: (i) Category I contains compounds with LD50 values ≤ 50 mg kg^{-1}, (ii) Category II contains compounds with LD50 values > 50 mg kg^{-1} but 500 mg kg^{-1} but 5000 mg kg^{-1} (Cheng *et al.,* 2012; Yang *et al.,* 2019). PROTOX is a Rodent oral toxicity server predicting LD_{50} value and toxicity class of query molecule. The toxicity classes are as follows: (Class 1: fatal if swallowed (LD50 ≤5), Class 2: fatal if swallowed (55000) (Banerjee *et al.,* 2018).

Docking of COVID-19 receptors and phytocompounds

The docking of selected ligands to the catalytic triad of protein was performed by using AutoDock/Vina (Trott and Olson, 2010). Docking was performed to obtain populations of conformations and orientation

for ligands at binding sites. Docking was performed to study the interactions between COVID-19 receptors 6VXX, 6VYB and receptor binding domain (2AJF) with major phytocompounds of *R. emodi*, *T. serpyllum* and *A. annua* and .pdb file of proteins-ligand complexes were generated. All bonds of ligands were set to be rotatable. All calculations for ligand flexible protein-fixed docking were performed using the Lamarckian Genetic Algorithm (LGA) method. The best conformation was chosen with the lowest docked energy, after the completion of docking search. The .pdb complex of protein and ligands were analyzed by PDBsum (www.ebi.ac.uk/pdbsum) to study the list of interactions between protein and ligands. Detailed visualization and comparison of the docked sites of target proteins and ligands were done by using Chimera (Pettersen *et al.,* 2004) and LigPlot (Laskowski and Swindells, 2011). Schematic representation experiments are shown in Fig. 2.

Results

ADMET prediction and toxicity analysis of selected phytocompounds

The comparative ADME properties predicted by SwissADME of selected phytocompounds and drug Chloroquine are summarized in Table 2. Consensus Log Po/w value of ≤5 indicates good aqueous solubility, which means that an adequate amount of drug can reach and be maintained inside the body through oral administration. All the selected phytocompounds have consensus Log Po/w value of < 5 (Table 2). TPSA indicates permeability of compounds into the cells. A TPSA value of < 140 Å2 is required for good permeation of compound into the cell membrane and value < 90 Å2 is required to permeate through blood–brain barrier. All the selected phytocompounds have TPSA value < 90 Å2, except 94.83 for emodin, indicating good permeability of selected phytocompounds through blood–brain barrier. Lipinski's rule of five helps to determine drug-likeness of the compound; an orally active drug should not violate the Lipinski's rule. All the ligands follow Lipinski's rule of five (Table 2) and active site of phytocompounds are shown in Table 3.

Table 2: ADME properties of selected phytocompounds and Chloroquine predicted by SwissADME.

	SwissADME								
Phytocompounds	Consensus Log PO/W	Water Solubility	GI Absorption	TPSA (Å2)	Lipinski Rule	Ghose Rule	Veber Rule	Egan Rule	Muegge Rule
Emodin	1.87	S	H	94.83	Y	Y	Y	Y	Y
Thymol	2.8	S	H	20.23	Y	No	Y	Y	No
Carvacrol	2.82	S	H	20.83	Y	No	Y	Y	No
Artemisnin	2.50	S	H	53.99	Y	Y	Y	Y	Y
Chloroquine	4.15	MS	H	28.16	Y	Y	Y	Y	Y

Y: Yes; H: High; S: Soluble; MS: Moderate Soluble

Table 3: Predicted targets of phytocompounds and standard drug chloroquine

Name of phytocompounds	Predicted Targets
Emodin	Estrogen receptor alpha, Estrogen receptor beta, Serine/threonine-protein kinase PIM1, Casein kinase II alpha, Protein-tyrosine phosphatase 4A3
Artemisinin	Cytochrome P450 1A2
Thymol	Transient receptor potential cation channel subfamily A member 1, Cyclooxygenase-1, GABA-A receptor; alpha-1/beta-2/gamma-2, Serotonin 2b (5-HT2b) receptor, GABA-A receptor; alpha-1/beta-3/gamma-2
Carvacrol	Cyclooxygenase-1, Transient receptor potential cation channel subfamily A member 1, Serotonin 2b (5-HT2b) receptor, Carbonic anhydrase II, GABA-A receptor; alpha-1/beta-3/gamma-2,
Chloroquine	Voltage-gated calcium channel alpha2/delta subunit 1, Serotonin 1a (5-HT1a) receptor, Histamine H3 receptor, Histamine N-methyltransferase , Alpha-1d adrenergic receptor

Toxicity of the phytocompounds and Chloroquine was predicted by PROTOX-II and admetSAR and results are summarized in Table 4. It was observed that all the selected phytocompounds are non-carcinogenic and non-cytotoxic in nature and are safe to administer. However, LD_{50} value of emodin and artemisinin calculated from Protox II was higher than that of all other phytocompounds and chloroquine, indicating that these natural phytocompounds are safer than that of chemically synthesized chloroquine.

Table 4: Toxicity prediction of phytocompounds and Chloroquine predicted by admetSAR and PROTOX-II software

Compounds	admet SAR		Protox II	
	Carcinogens	Rate Acute toxicity (LD_{50}) kg/mol	LD_{50}, (mg/kg)	Cytotoxicity
Emodin	non-carcinogen	2.5826 (III)	5000 (Class 5)	Inactive
Thymol	non-carcinogen	2.202 (III)	640 (Class 4)	Inactive
Carvacrol	non-carcinogen	2.531 (III)	1190 (Class 4)	Inactive
Artemisinin	non-carcinogen	1.79 (V)	4228 (Class 5)	Inactive
Chloroquine	non-carcinogen	2.684 (II)	311 (Class 4)	Inactive

Molecular Docking analysis of phytocompounds and Chloroquine with spike receptors and receptor binding domain of COVID-19

Docking results with selected molecular targets of covid-19 spike protein i.e. SARS-CoV-2 spike glycoprotein (6VXX), SARS-CoV-2 spike ectodomain structure (6VYB), and SARS coronavirus spike receptor binding domain (2AJF) showed that selected phytocompounds had a good binding affinity and better binding modes than that of standard drugs chloroquine. All the 4 phytocompounds the best binding affinity, among all the phytocompound artemisinin (-10.5 kcal mol^{-1}) showed the best followed by thymol (-6.9 kcal mol^{-1}), carvacrol (-6.8 kcal mol^{-1}), emodin (-6.4 kcal mol^{-1}) and chloroquine (-5.6 kcal mol^{-1}) with SARS-CoV-2 spike glycoprotein (6VXX) (Table 5, fig. 3 and S1).

In case of SARS-CoV-2 spike ectodomain structure (6VYB) artemisinin (10.3 kcal mol^{-1}) showed best binding affinity followed by emodin (-8.8 kcal mol^{-1}), carvacrol (-6.8 kcal mol^{-1}), thymol (-6.7 kcal mol^{-1}) and chloroquine (-5.9 kcal mol^{-1}) (Table 5 and fig. 4 and S2).

In case of SARS coronavirus spike receptor binding domain (2AJF) artemisinin (-9.1 kcal mol^{-1}) followed by carvacrol (7.4 kcal mol^{-1}), thymol (-7.2 kcal mol^{-1}), emodin (6.4 kcal mol^{-1}) and chloroquine (6.4 kcal mol^{-1}) (Table 5, fig. 5 and S3).

Furthermore, nature of hydrogen bonds was determined on the basis of donor-acceptor distances in protein secondary structure elements. Jeffrey and Jeffrey (1997) categorizes hydrogen bonds with donor-acceptor distances of 2.2-2.5 Å as "strong, 2.5-3.2 Å as "moderate (mostly electrostatic), and 3.2-4.0 Å as "weak, electrostatic". Hydrogen bond interaction, and hydrophobic interactions of these phytocompounds with all receptors were analyzed through Ligplot and summarized in table 5.

Table 5: E-total of ligands (emodin, thymol, carvacrol artemisnin and chloroquine) with COVID-19 targets by using Autodock vina software.

Receptor	Ligands	E_{total} (kcal mol^{-1})	Interacting amino acids	
			H-bonding	Hydrophobic interaction
SARS-CoV-2 spike glycoprotein (6VXX)	Emodin	-6.4	SER 1003, ARG 1014 (weak)	GLN 1010, ALA 958, THR 1006, TYR 1007, THR 961, GLN 965
	Thymol	-6.9	-	NO INTRACTION
	Carvacrol	-6.8	HIS 1058, ALA 1056 (moderate)	THR 778, GLY 1059, PHE 782, ILE 870, LEU 865, PRO 863, SER 730
	Artemisnin	-10.5	THR 778, SER 730 (week)	LEU 865, PHE 782, ASP 867, PRO 863, HIS 1058, ILE 870. ALA 1056GLY 1059, VAL 729
	Chloroquine	-5.6	VAL 826 (weak)	THR 778, SER 730, PRO 863, ALA 1056, ILE 870, ASP 867, LEU 867, HIS 1058, PRO 1057, PHE 823, THR 827
SARS-CoV-2 spike ectodomain structure (6VYB)	Emodin	-8.8	THR 778, SER 730 (weak)	VAL860, LEU 861, THR 732, LYS 733, PRO 863, MET 731, HIS 1058, ALA 1056, ASP 867, PHE 823
	Thymol	-6.7	SER 730, THR 778 (moderate)	LEU 865, PRO 863, PHE 782, ILE 870, ALA 1056, GLY 1059
	Carvacrol	-6.8	SER 730, THR 778 (moderate)	VAL 729, PHE 782, ILE 870, ASP 867, ALA 1056, GLY 1059, LEU 865
	Artemisnin	-10.3	SER 730, THR 778 (weak)	HIS 1058, ALA 1056, GLY 1059, VAL 729, PRO 863, LEU 865, PHE 782, ILE 870, ASP 867
	Chloroquine	-5.9	SER 730, HIS 1058 (weak)	PRO863, PRO 1057, ALA 1056, ASP 867, THR 827
SARS coronavirus spike receptor-binding domain (2AJF)	Emodin	-6.9	TYR 385 (weak)	HIS 401, HIS 378, ASP 382, ALA 348, ASP 350, TRP 349, PHE 40
	Thymol	-7.2	-	GLU 232, PRO 235, LEU 236, PHE 592, GLU 589, LEU 585
	Carvacrol	-7.4	GLU 589 (moderate)	LEU 236, GLU 232, PHE 592, LEU 585, PRO 235
	Artemisnin	-9.1	-	PHE 390, ARG 393, ASP 350, PHE 40
	Chloroquine	-6.4	ASN 394 (moderate)	LEU 73, ALA 99, LEU 100, TRP 69, PHE 390, ARG 393, ASP 350, PHE 40

Discussion

From the beginning of 21st century, three corona viruses have crossed the species barrier and resulted in deadly pneumonia in humans: severe acute respiratory syndrome coronavirus (SARS-CoV) (Ksiazek*et al.,* 2003), Middle-East respiratory syndrome coronavirus (Zaki*et al.,* 2012) (MERS-CoV) and SARS-CoV-2 (Huang *et al.,* 2020; Zhu *et al.,* 2020). Nowadays, SARS-CoV-2 has been caused death of approximately 75,000 people all around the world. There is no specific antiviral treatment recommended for COVID-19, and no vaccine is currently available. The treatment is symptomatic, and oxygen therapy represents the major treatment intervention for patients with severe infection. Mechanical ventilation may be necessary

in cases of respiratory failure refractory to oxygen therapy, whereas hemodynamic support is essential for managing septic shock. Researchers from worldwide are continuing to work on developing vaccine against Covid-19. Professor Didier Raoult from infectious diseases institute, IHU Méditerranée Infection in Marseille (France) has reported successful results from a new treatment for Covid-19, with early tests suggesting it can stop the virus from being contagious in just six days. Chloroquine phosphate and hydroxychloroquine have previously been used to treat coronavirus patients in China, in ongoing COVID-19 clinical trials. Kaletra, a US-based antiviral drug used to treat HIV, is another medicine that is being tested in the fight against the deadly virus. Emodin act as inhibitor of 3a ion channel of corona virus SARS-CoV and HCoV-OC43 as well as virus release from HCoV-OC43. They reported emodin is a potent inhibitor of the 3a channel with a K1/2 value of about 20 M. The reduction of extracellular viral RNA copies by emodin reflects inhibition of virus release. At high concentrations of emodin also intracellular levels of viral RNA copies were reduced suggesting that the high concentrations may also inhibit other stages of the virus life cycle (Schwarz *et al*. 2011). Ho *et al*. (2007) identified emodin as an effective to block the interaction of the SARS-CoV S protein with the ACE2 and the infection by S protein-pseudo-typed retrovirus. Similar to our study, Kumar *et al* (2020) reported the binding affinity of Nelfinavir (-8.4), Rhein (-8.1), Withanolide D (-7.8), Withaferin A (-7.7), Enoxacin (-7.4), and Aloe-emodin (-7.4) with COVID 19 main protease (6LU7).

Conclusions

Present study proposed safe and less toxic phytocompounds for the treatment for COVID-19, which can be further validated through *in vitro* and *in vivo* studies.

Declarations

Contribution: All the experimental work was done by Er Rajan Rolta and Ms. Deeksha Salaria. Dr Vikas Kumar provided the technical inputs. Dr Anuradha Sourirajan and Dr Kamal Dev conceived the idea and provided guidance to execute the research project. All the authors have read the manuscript.

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Figures

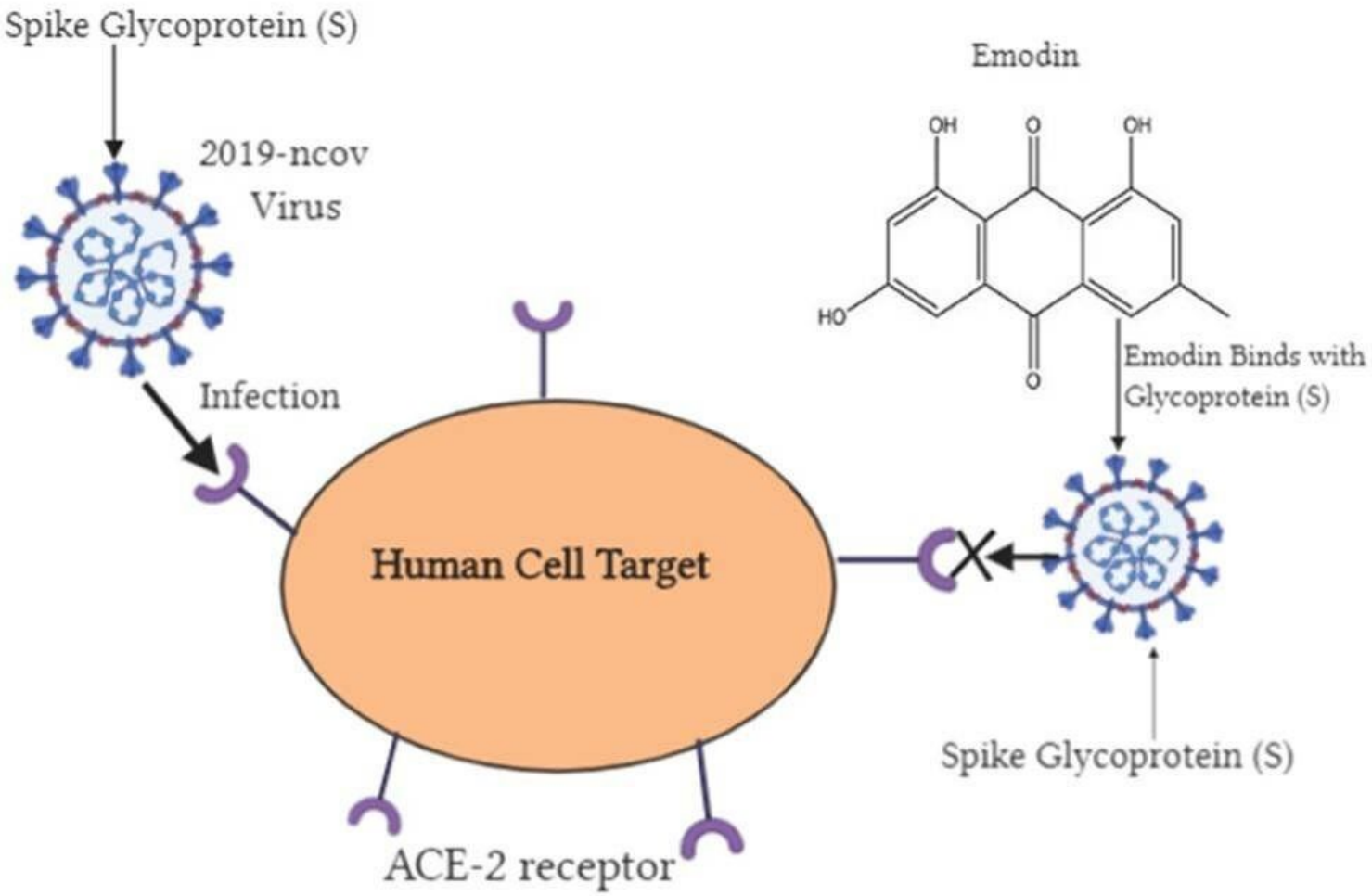

Figure 1

Pictorial representation of binding of Covid-19 with ACE-2 receptor and prevention of this binding using phytocompounds (emodin).

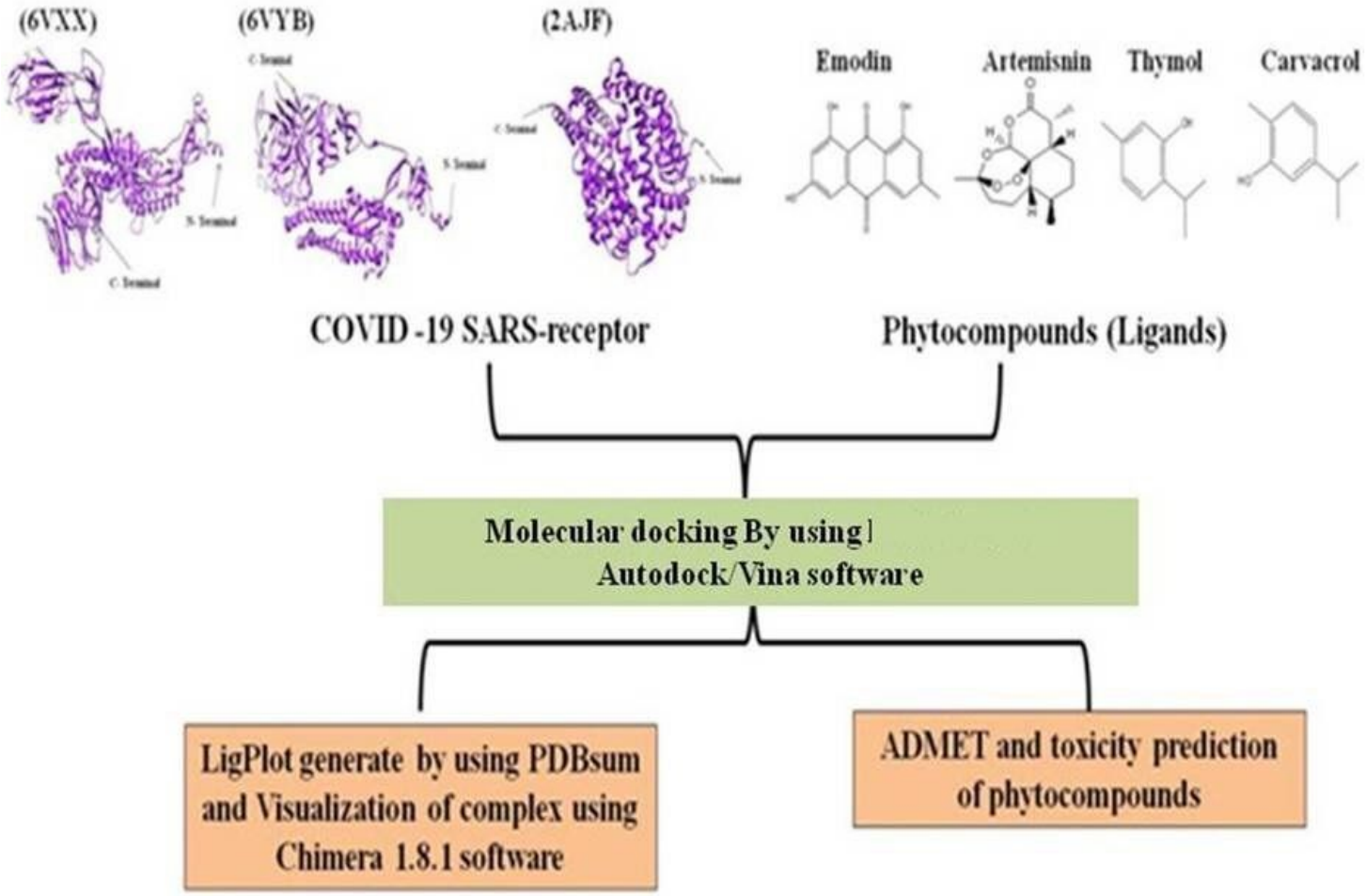

Figure 2

Schematic representation of process adapted for the present study

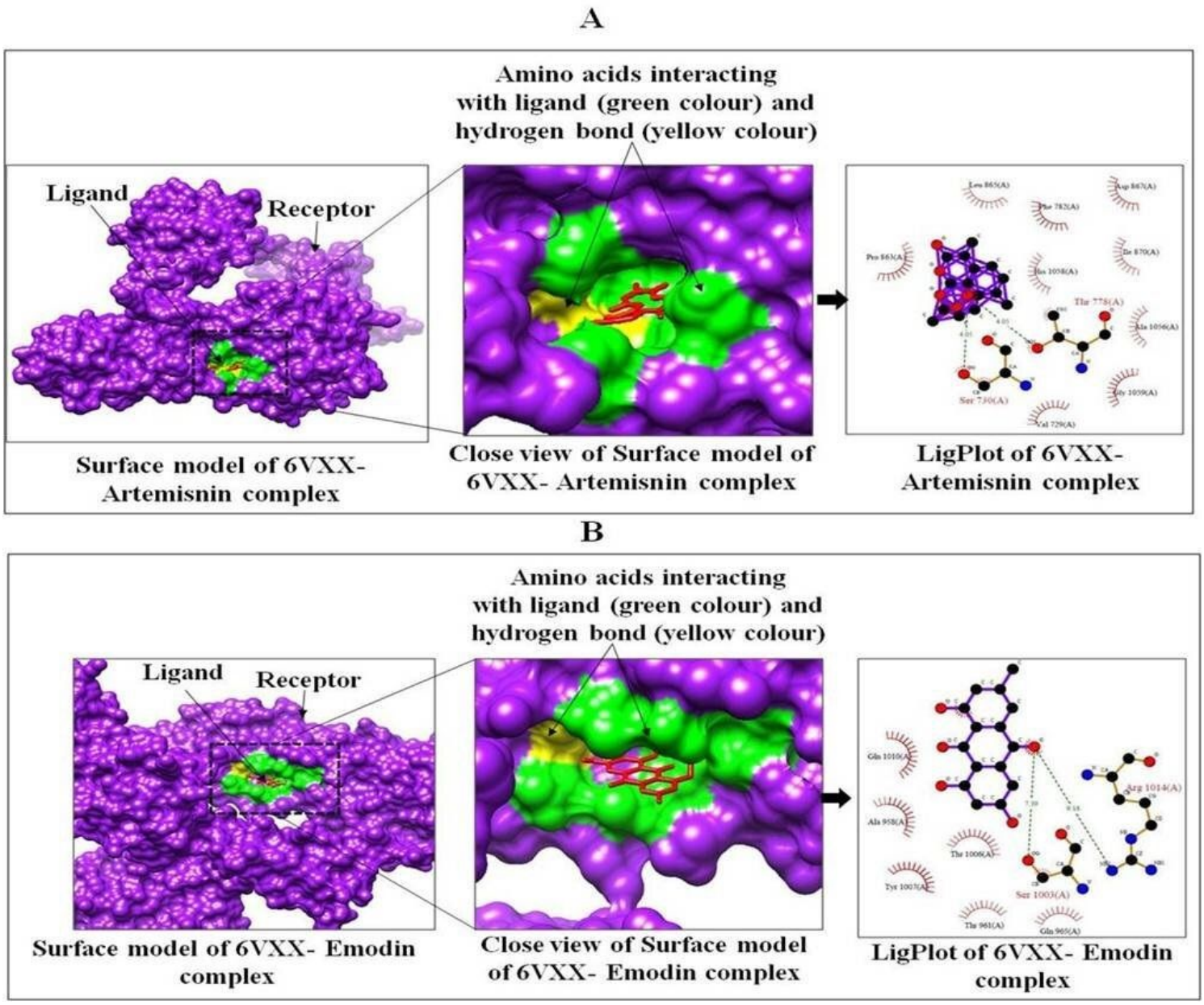

Figure 3

Interactions between targeted protein receptor SARS-CoV-2 spike glycoprotein (PDB ID: 6VXX) with Artemisinin (A), Emodin using Chimera and LigPlot analysis.

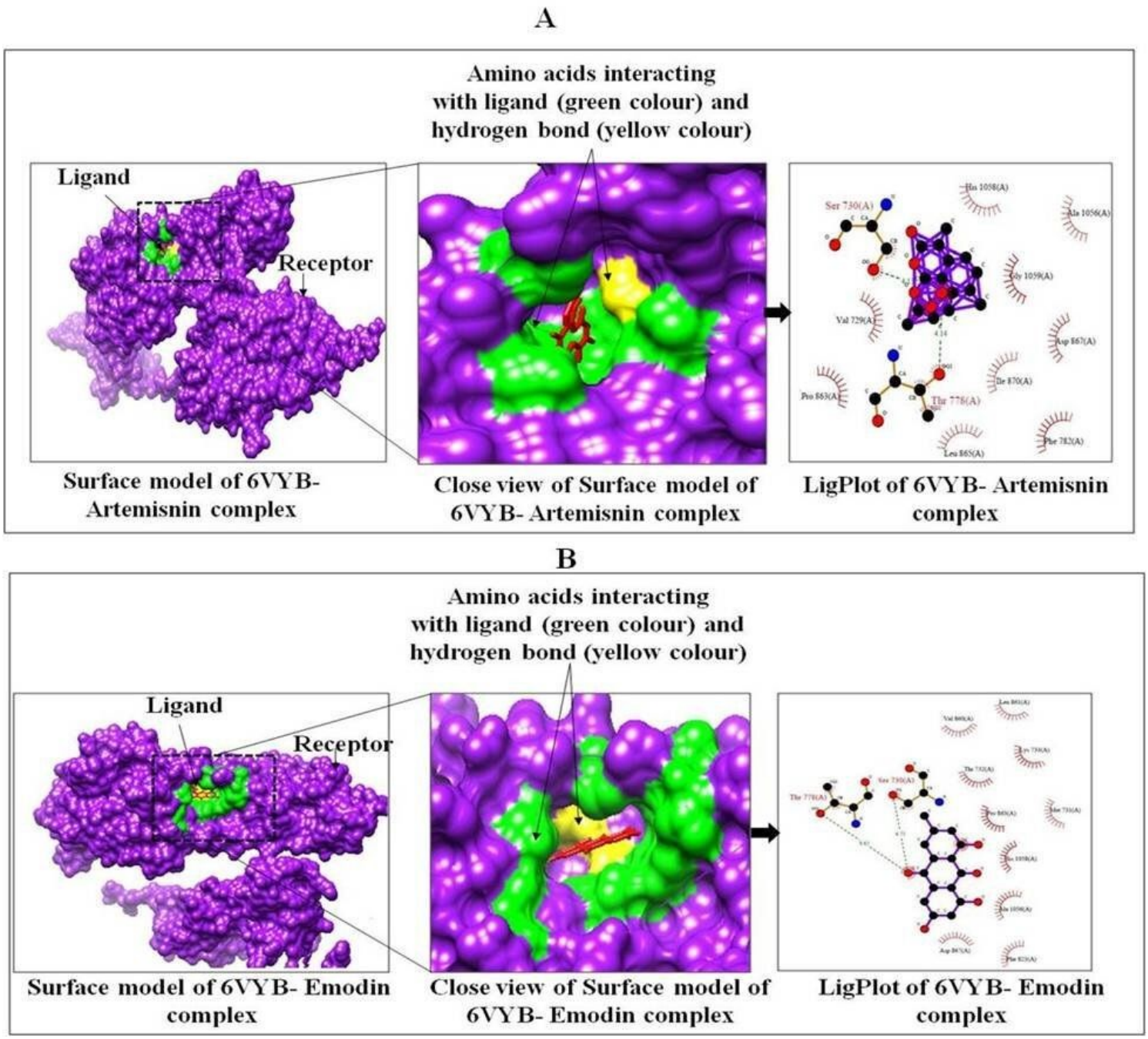

Figure 4

Interactions between targeted protein receptor SARS-CoV-2 spike ectodomain structure (PDB ID: 6VYB) with Artemisinin (A), Emodin (B) using Chimera and LigPlot analysis.

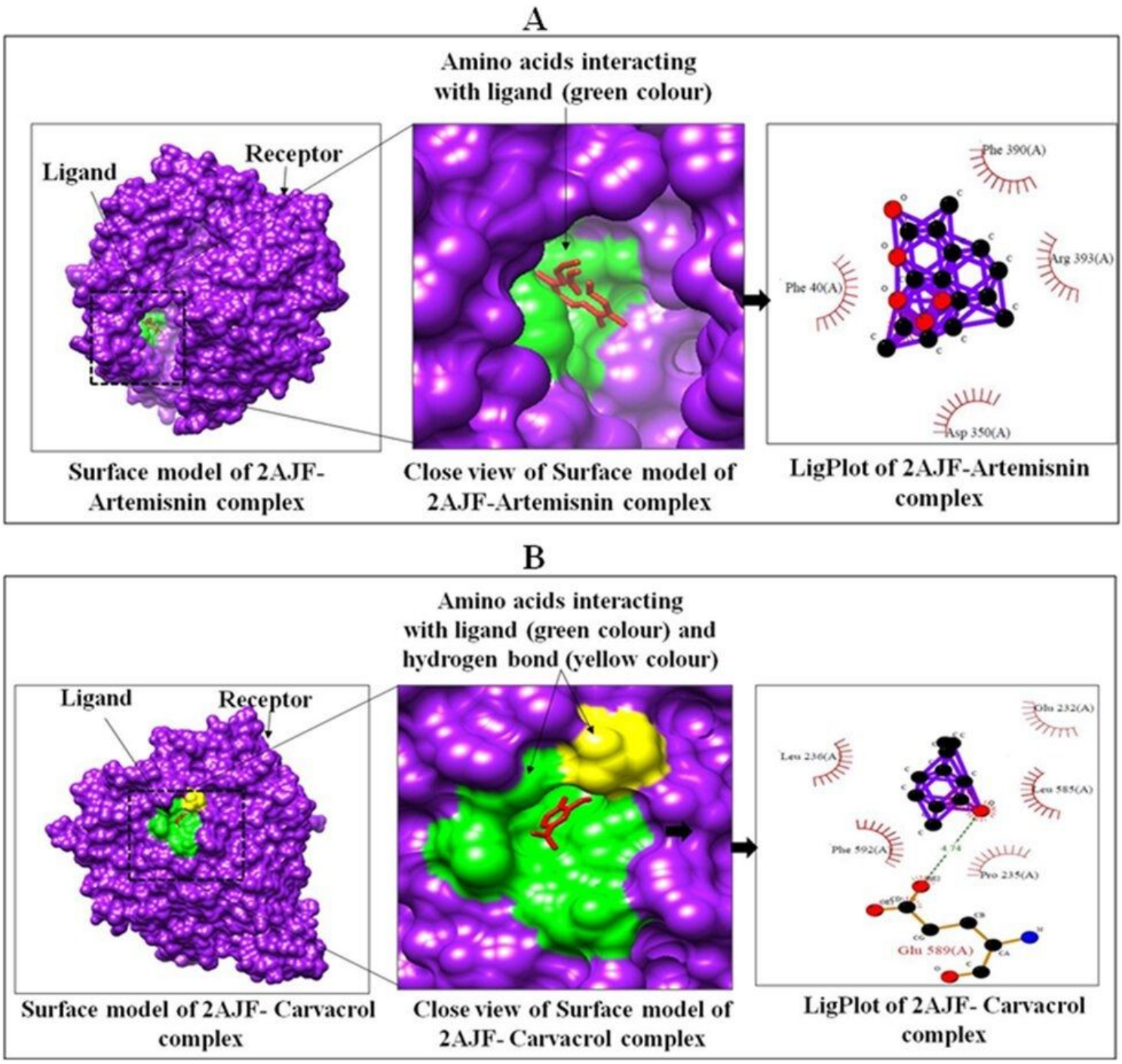

Figure 5

Interactions between targeted protein receptor SARS coronavirus spike receptor-binding domain (PDB ID: 2AJF) with Artemisinin (A), Emodin using Chimera and LigPlot analysis.

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