Evaluation of traditional ayurvedic preparation for prevention and management of the novel Coronavirus (SARS-CoV-2) using molecular docking approach

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Abstract

Since the emergence of novel Coronavirus (SARS-CoV-2) infection in Wuhan, China in December 2019, it has now spread to over 205 countries. The ever-growing list of globally spread corona virus-19 disease (COVID19) patients has demonstrated the high transmission rate among human population. Although 12 new drugs are being tried for management of COVID19, currently there are no FDA approved drugs or vaccines to prevent and treat the infection of the SARS-CoV-2. Considering the current state of affairs, there is an urgent unmet medical need to identify novel and effective approaches for prevention and treatment of COVID19 by re-evaluating the knowledge of traditional medicines and repurposing of drugs. Here, we used molecular docking approach to explore the beneficial roles of an array of phytochemicals and active pharmacological agents present in the Indian herbs (Tulsi, Haldi, Giloy, Black pepper, Ginger, Clove, Cardamom, lemon, and Ashwagandha) which are widely used in the preparation of Ayurvedic medicines in the form of *Kadha* to control various respiratory disorders such as cough, cold and flu. The evaluation was made based on the docking scores calculated by AutoDock Vina. Our study has identified an array of phytochemicals present in these herbs which have significant docking scores and potential to inhibit different stages of SARS-CoV-2 infection as well as other Coronavirus target proteins. Molecular docking also indicated that, the phytochemicals present in these herbs possess significant anti-inflammatory property. Overall our study provides scientific justification in terms of binding of active ingredients present in different plants used in *Kadha* preparation with viral proteins and target proteins for prevention and treatment of the COVID19. This preparation can boost individual's immunity and inhibit the viral severity by interfering at different stages of virus multiplication in the infected person.

Introduction

Viral infections cause a wide spectrum of human diseases which appear with mild, severe or life-threatening symptoms and underlie major clinical and socio-economic problems worldwide. Human Coronaviruses (CoVs), including severe acute respiratory syndrome Coronavirus (SARS-CoV), middle east respiratory syndrome Coronavirus (MERS-CoV) and 2019 novel Coronavirus (SARS-CoV-2) have caused three recent major global epidemics with

significant morbidity and mortality (Paules et al., 2020). Presently, there are no specific drugs or vaccines approved for SARS-CoV-2. In light of the high infectivity rate of SARS-CoV-2 and lack of treatment options, World Health Organization (WHO) has declared it as a global emergency and researchers from all-over the world are trying to find possible cure for this disease called Corona Virus Disease or COVID19. Phylogenetic analysis shows that SARS-CoV-2 has very high nucleotide sequence identity with SARS-CoV-1 (79.7%) (Zhou et al., 2020). The envelope and nucleocapsid proteins of SARS-CoV-2 are two evolutionarily conserved regions, with sequence identities of 96% and 89.6%, respectively. CoVs are enveloped viruses with a positive-sense single-stranded RNA genome and contain at least four structural proteins: Spike (S) protein (trimeric), envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein. The Spike protein promotes host attachment and virion-cell membrane fusion during infection. Therefore, Spike proteins play a crucial role in determining the host range and tissue tropism. Zoonosis is common among CoVs and they can be transmitted from one animal species to another, animals to humans, and humans to humans (Lim et al., 2019).

Therapeutic interventions against CoVs can either activate the host defence machinery and immune system or block viral life cycle events including transmission, cell binding, enzymes involved in synthesis of the viral components, replication and assembly (Wu et al., 2020). In search of therapeutics against CoVs, researchers are using following three broad strategies; (i) Test existing broad-spectrum anti-viral drugs, (ii) in silico screening of molecular databases to identify the lead molecules against viral or host proteins and (iii) rational drug design based on the genomic information and pathological characteristics of COVID19 (Wu et al., 2020). Among these, repurposing approach will shorten the time and reduce the cost as compared to other strategies (Wu et al., 2020). Apart from the above strategies, alternative approaches including traditional and herbal medicines may also have significant potential for management of COVID19 both as prophylaxis and therapeutic purpose.

Ayurvedic medicines and their extracts are used for treatment of viral diseases for a very long time (Arora et al., 2010; Alleva et al., 2010). Ayurvedic medicines are used in Indian subcontinent since the Vedic period dating back to more than 2000 years (Saini, 2016). An important Ayurvedic method for enrichment of active pharmacological agents from herbs involves preparation of *Kadha (*decoction) for oral consumption. According to *Panchvidh Kashyapam* described in the Charak Samhita (an ancient text on Ayurveda), there are five

prescribed ways to consume medicinal herbs and plants (Shingadiya et al., 2016). These includes: i) Swaras (juicing), ii) Kwath (decoction), iii) Kalka (in paste form), iv) Hima (an herb induced concoction), v) Phant (an herb-infused concoction). The decoction (*Kadha*) from a mixture of spices and herbs is considered to be one of the oldest forms of medicine invented by humans. *Kadha* is prepared from dry or less juicy ingredients like spices and herbs. The Ministry of AYUSH (Ayurvedic, Yoga and Naturopathy, Unani, Siddha and Homeopathy), Government of India, has recently recommended the use of Kadha for boosting the immunity and reducing inflammation during COVID 19 crisis (The ministry of AYUSH, 2020).

During viral infections, inflammation is part of the body's immune response to reduce infection, limit viral replication and transmission, reduce tissue injury and kill infected cells. Acute inflammation is beneficial and it is followed by healing and regeneration. However, CoVs are known to manipulate host machinery and subvert the immune system leading to chronic inflammation (Chen et al., 2017; Takeuchi and Akira, 2007). The induction of pro-inflammatory cytokines and chemokines in the host during SARS-CoV infection acts as a double-edged sword which not only activates host immune response for viral clearance but also aggravates tissue injury and organ toxicity during clinical evolution of the disease (Gu and Korteweg, 2007; Tisoncik et al., 2012). CoVs infect cells using their spike proteins by making interaction with cognate receptors present on the host cell surface. The spike proteins of SARS-CoV and MERS-CoV attach to the cellular receptor angiotensin-converting enzyme 2 (ACE2) and dipeptidyl peptidase 4 (DPP4) receptor respectively for their entry inside the cells (Gu and Korteweg, 2007; Wang et., 2013). SARS-CoV-2 spike protein has higher affinity towards human ACE2 as compared to that of SARS-CoV-1 (Zhou et al. 2020). Upon infecting the host cells, CoVs manipulate host machinery for new virus production and also elicit inflammatory response in the host due to tissue injury (Gu and Korteweg, 2007; Wang et., 2013). Clinical investigation of critically ill patients infected with SARS-CoV-2 have shown high concentration of cytokines and chemokines in human plasma, suggesting that cytokine storm was associated with disease severity, multi-organ failure and mortality (Huang et al., 2020). Cyclooxygenase-2 (COX-2), phospholipase A2 (PLA2), NF-κB-inducing kinase (NIK), and interleukin-1 receptor associated kinase (IRAK) are important druggable targets involved in SARS-CoV-2 induced inflammatory response and can be used to screen anti-inflammatory molecules.

In the present study, the phytochemicals and active pharmacological ingredients found in different herbs used in making *Kadha* were docked with different viral proteins (such as viral capsid spike, proteases, NSP polymerase), host cell receptors & proteases (such as human ACE2 and furin) as well as pro-inflammatory mediators (such as COX2, PLA2, IRAK-4 and NIK proteins). Our study predicted that many of these phytochemicals possess significant affinity towards functional region of viral proteins including spike, proteases, nucleoproteins and polymerase as well as host surface receptors. These phytochemicals also showed significant binding affinity for the functional region of different inflammatory mediators. Our findings indicate that regular consumption of this ayurvedic *Kadha* in consultation with the ayurvedic practitioner may significantly boost the host immunity and also help in the prevention of viral infection and pathogenicity and reduce disease-severity in the infected individuals.

Materials and Methods

Phytochemicals name of commonly used herbs in making *Kadha*: Some of the commonly used herbs in the preparations of *Kadha* includes, Tulsi (*Ocimum sanctum*), Haldi (*Curcuma longa*), Giloy (*Tinospora cordiofolia*), Black pepper (*Piper nigrum*), Ginger (*Zingiber officinale*), Clove (*Syzygium aromaticum*), Cardamom (*Elettaria cardamomum*), lemon (*Citrus limon*), and Ashwagandha (*Withania somnifera*). A list of 108 phytochemicals present in herbs that are used in preparation of *Kadha* or similar drink were collected from literature. Phytochemicals found in *Ocimum sanctum* (Oleanolic acid, Ursolic acid, Rosmarinic acid, Eugenol, Carvacrol, Linalool, Beta-caryophyllene, Estragole, Eugenic acid, Apigenin, Cirsimaritin, Isothymusin, Isothymonin, Vicenin, Orientin and Cirsilineol) (Pattanayak et al., 2020), *Curcuma longa* (Curcumin, Demethoxycurcumin, Bisdemethoxycurcumin, Ar-turmerone, Alpha-turmerone, Beta-turmerone, Atlantone, Cyclocurcumin, Calebin A , Trans-Ferulic acid, Vanillin and Vanillic acid) (Li et al., 2011), *Tinospora cordiofolia* (Magnoflorine, Berberine, Choline, Jatrorrhizine, Beta-Sitosterol, Tinosporide, Tinosporaside, Cordifolioside A, Tinocordioside, Cordioside, Tinocordifolioside andTinocordifolin) (Sharma et al., 2019), *Piper nigrum* (Piperine, Piperamide, Piperamine, Pipericide, Sarmentosine, Sarmentine, Brachyamide B, Dihydropipericide, N-Formylpiperidine, Guineensine, Pentadienoylpiperidine, Tricholein, Trichostachine, Piperettine, Piperolein B, Retrofractamide A, Chavicine, Isochavicine,

Isopiperine, Nerolidol, β-caryophyllene and Piperic acid) (Damanhouri and Ahmad, 2014), *Zingiber officinale* (6-gingerol, 6-shogaol, 6-paradol, Zingiberene, Bisabolene, 1-dehydrogingerdione, 6- gingerdione, 10-gingerdione, 4-gingerdiol, 6-gingerdiol, 10-gingerdiol, Citral and Eucalyptol)(Bhattarai et al., 2018; Prasad et al., 2015), *Syzygium aromaticum* (beta-caryophyllene, Vanillin, Eugenol, Acetyl eugenol, Crategolic acid, Eugenin, Methyl salicylate, Kaempferol, Rhamnetin, Eugenitin, Oleanolic acid, Stigmasterol, Campesterol, Gallic acid and Flavonol glucosides) (Cortés-Rojas et al., 2014), *Elettaria cardamomum* (Protocatechualdehyde, Protocatechuic acid, Alpha-terpinyl acetate, 1,8-cineole, Linalool, Linalyl acetate, Limonene, 4-terpineol andGeraniol) (Noumi et al., 2018), Citrus limon (Eriodictyol, Quercetin, Hesperetin, Phloroglucinol, Umbelliferone, Vitamin C (Vandercook and Stephenson, 1966; Rangel et al., 2011) and *Withania somnifera* (Withaferin A, Somniferine, Choline, Anaferine, Withanolide A, Withanolide B, Withanone and Withanolide) (Sangwan et el., 2004). 3D structures of these different phytochemicals were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov) in structure-data file (SDF).

Preparation of Ayurvedic *Kadha*: There are specific Ayurvedic methods to prepare the *Kadha*. In India, variants of standard *Kadha* are also prepared using different combinations of herbs depending on the severity of disease / ailment and availability of ingredients. The most common ingredients are Tulsi leaves (10-15 leaves or ¼ teaspoon powder), Ginger (2-5g rhizome or ¼ teaspoon powder), Clove (4-5 pieces), Black pepper (4-5 pieces), Cardamom (4-5 pieces), Ashwagandha (2-5g raw or ¼ teaspoon powder) and Giloy (2-5g raw or ¼ teaspoon powder). To make the *Kadha,* these herbs are boiled in 200 ml water for 5-10 minutes, and jaggery or honey is added to make it sweet. The preparation is filtered and mixed with ¼ teaspoon of lemon juice. In case, if all ingredients are not available, it can be prepared using locally available ingredients.

Protein structures: In order to study the mode of interaction of different phytochemicals with various proteins including SARS-CoV-2, SARS-CoV-1, other important proteins and receptors found on virus and host cells, molecular docking was performed. We have used following PDB ID's 6LU7 (SARS-CoV-2 main protease), 6m3m (SARS-CoV-2 nucleocapsid), 6vww (SARS-CoV-2-NSP15 Endoribonuclease), 6vyo (SARS-CoV-2 RNA binding domain), 6w02 (SARS CoV-2-NSP3), 6w4b(SARS-CoV-2-NSP9 replicase), 2ajf (ACE2 and SARS-CoV spike), 4mds (SARS-CoV 3CLpro) and 5mim (proprotein convertase; furin). In order to study the interaction of different

phytochemicals with different pro-inflammatory mediators like COX2, PLA2, NIK and IRAK-4, we have used following PDB ID's, 5f1a, 4uy1, 4dn5 and 2nrurespectively. All the protein structures were retrieved from protein data bank (www.rcsb.org) and cleaned using UCSF Chimera, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California.

Molecular docking: PyRx virtual screening tools was used for preparation of the input files and performing molecular docking using Vina wizard. For preparation of protein input files, all water molecules, ligands and ions were removed from *.pdb files. The polar hydrogens were added to protein structure and prepared files were saved in *.pdbqt format. The molecule's energy was minimized using energy minimization tools of PyRx virtual screening tools and ligands were saved in *.pdbqt format after adding polar hydrogens for further docking process. All docking results were sorted by the binding energy. 2D interaction of the ligand and protein was visualized using Discovery Studio Visualizer. Region-specific docking was performed against SARS-CoV-2 main protease and spike protein as well as for human ACE2 & Furin protease. Following AutoDock Vina docking parameters such as (center_x = -16.69, center_y = 27.23, center_z = 68.46, size_x = 36.65, size_y = 42.12, size_z = 50.40), (center_x = 190.45, center_y = 197.88, center_z = 260.72, size_x = 61.32, size_y = 41.03, size_z = 43.79), (center_x = 6.68, center_y = -2.42, center_z = 48.2, size_x = 37.03, size_y = 70.29, size_z = 55.72) and (center_x = 32.41, center_y = -37.97, center_z = -11.64, size_x = 71.93, size_y = 55.05, size_z = 47.46) were used for SARS-CoV-2 main protease (PDB ID: 6LU7), SARS-CoV-2 spike (PDB ID: 6VXX), human ACE2 (PDB ID: 2AJF) and furin (PDB ID: 5MIM) respectively. Other proteins from SARS-CoV-2, SARS-CoV and host anti-inflammatory mediators were blindly docked using complete protein structure.

Results

Our molecular docking study revealed that different phytochemicals found in the traditional Ayurvedic *Kadha,* may have high binding affinity (the lowest binding energy) with various viral and host macromolecular targets and other human pro-inflammatory mediators and proteins. Table 1 shows the list of the phytochemicals which showed significant binding

affinity (≤-7.5 kcal/mole) with SARS-CoV-2 main protease, spike protein, human ACE2 and furin proteins.

Table 2 shows the predicted binding of top 12 phytochemicals and native ligand N3, against SARS-CoV-2 main protease and their different protein residues which are involved in the interaction. These molecules are Somniferine A, Tinosporide, Tinocordioside, Orientin, Flavonol glucoside, Withanolide, Apigenin, Cyclocurcumin, Withanolide B, Kaempferol, Withanone and Withaferin A. Their predicted binding energies are -8.6, -8.6, -8.1, -8.1, -8.0, -8.0, -7.8, -7.8, -7.8, -7.7, -7.6 and -7.6 kcal/mole respectively. All these molecules share same binding site with the well-known inhibitor, N3 and their binding energy is also comparable (Fig 1).

SARS-CoV-2 spike protein interacts with the host ACE2 receptor present on the surface of the host cells for their entry. Table 3 shows the binding of top 12 phytochemicals with SARS-CoV-2 spike (PDB ID: 6VXX) and the different residues involved in their interaction. These molecules are, Withanolide B, Withanolide, Withaferin A, Withanone, Somniferine A, Beta-sitosterol, Pipericide, Crategolic acid, Retrofractamide A, Ursolic acid, Piperolein B and Tinosporide. Their predicted binding energies are -8.7, -8.4, -8.2, -8.2, -7.9, -7.8, -7.6, -7.6, -7.5, -7.5, -7.5, -7.5, -7.4 and -7.4 kcal/mole respectively. These phytochemicals were found to share same binding pocket in the target proteins (Fig 2 a).

Table 4 shows the binding of top 10 phytochemicals with human ACE2 (PDB ID: 2AJF) and the different residues involved in their interaction. The phytochemicals with high affinity for human ACE2 are, Withaferin A, Stigmasterol, Vicenin, Ursolic acid, Withanolide B, Oleanolic acid, Withanone, Beta-sitosterol, Campesterol and Orientin. Their predicted binding energies are -9.1, -8.8, -8.8, -8.7, -8.7, -8.5, -8.5, -8.3, -8.3 and -8.0 kcal/mole respectively. These phytochemicals share same binding pocket in human ACE2 (Fig 2 b).

Furin is another protease found in the host cells which acts on the viral spike protein and facilitates its interaction with the human ACE2. Table 5 shows the predicted binding of top 12 phytochemicals with human furin (PDB ID: 5MIM) and the different residues involved in their interactions. These molecules are, Withanolide, Somniferine A, Apigenin, Withanolide B, Ursolic acid, Hesperetin, Campesterol, Crategolic acid, Withanone, Chavicine, Rosmarinic acid and Stigmasterol. Their predicted binding energies are -9.5, -8.7, -8.5, -8.3, -8.3, -8.2, -8.1, -

8.1, -8.1, -8.0, -8.0 and -8.0 kcal/mole respectively. Many of these molecules share same binding pocket as its native ligand. However, our docking study also showed that Apigenin, Withanolide, Hespertin, Campesterol, Chavicine, Rosmarinic acid, Stigmastereol bind to the residues at a site adjacent to binding site of native ligand (Fig 3).

The molecular docking performed against other proteins from SARS-CoV-2 and SARS-CoV-1proteins are shown in Table 6. Many of the phytochemicals present in the *Kadha* have significant binding affinity with SARS-CoV-2 and SARS-CoV-1 proteins. Some of the phytochemicals which have high binding affinity with NSP15 Endoribonuclease (PDB ID: 6vww) are Orientin, Withanolide, Withanolide B, Crategolic acid, Ursolic acid, Withaferin A, Apigenin, Eriodictyol, Hesperetin, Oleanolic acid, Stigmasterol and Withanone. Their predicted binding energies are -9.4, -9.3, -9.2, -9.1, -9.1, -9.1, -9.0, -9.0, -9.0, -9.0, -8.9 and -8.9 kcal/mole respectively. A list of the phytochemicals which were found to exhibit high affinity with SARS-CoV-2 ADP ribose phosphatase, NSP3 (PDB ID: 6w02) are Berberine, Withanolide, Rosmarinic acid, Cyclocurcumin, Piperettine, Withaferin A, Withanolide B, Bisdemethoxycurcumin, Chavicine, Cirsimaritin, Demethoxycurcumin and Apigenin. Their predicted binding energies are -9.7, -9.6, -9.3, -9.2, -9.2, -9.2, -9.2, -8.8, -8.8, -8.8, -8.8, and -8.7 kcal/mole respectively. Among the phytochemicals tested, the following molecules were predicted to bind with Nsp9 RNA binding protein of SARS CoV-2 (PDB ID: 6w4b) protein, Withanone, Withanolide B, Withaferin A, Withanolide, Beta-sitosterol, Campesterol, Stigmasterol, Flavonol glucoside and Somniferine A. Their predicted binding energies are -9.1, -9.0, -8.2, -8.1, -8.0, -8.0, -7.9, -7.5 and -7.5 kcal/mole respectively.

More than ten phytochemicals were predicted to interact with SARS-CoV-2 RNA binding domain of nucleocapsid protein (PDB ID: 6vyo) viz, Withanolide, Withaferin A, Somniferine A, Withanone, Withanolide B, Ursolic acid, Crategolic acid, Quercetin, Stigmasterol, Tinocordioside, Tinosporaside and Oleanolic acid. Their predicted binding energies are -8.7, -8.3, -8.2, -8.2, -8.0, -7.8, -7.7, -7.7, -7.7, -7.7, -7.7 and -7.6 kcal/mole respectively.

An array the phytochemicals (having binding energy ≤ 7.5 kcal/mole) were also predicted to interact with SARS-CoV main protease (PDB ID: 4mds) viz, Somniferine A, Withanolide B, Withanone, Withanolide, Oleanolic acid, Cordioside, Crategolic acid, Tinosporaside, Calebin A, Ursolic acid, Stigmasterol, Orientin, Pentadienoylpiperidine, Campesterol, Cyclocurcumin, Rosmarinic acid, Tinosporide, Withaferin A, Demethoxycurcumin, Jatrorrhizine, Piperettine,

Tinocordioside and Trichostachine. Their predicted binding energies are -9.3, -8.9, -8.5, -8.3, -8.2, -8.1, -8.1, -8.1, -8.0, -8.0, -7.9, -7.8, -7.8, -7.7, -7.7, -7.7, -7.7, -7.7, -7.6, -7.5, -7.5, -7.5 and -7.5 kcal/mole respectively. Similarly, the phytochemicals (having energy ≤7.5 kcal/mole) which may interact with SARS-CoV-1 spike protein (PDB ID: 2ajf) include, Withanolide, Stigmasterol, Withanolide B, Somniferine A, Ursolic acid, Beta-sitosterol, Oleanolic acid, Pentadienoylpiperidine, Piperettine, Tinosporide, Withanone, Bisdemethoxycurcumin, Calebin A, Campesterol, Cyclocurcumin, Piperine, Retrofractamide A, Trichostachine and Quercetin. Their predicted binding energies are -8.7, -8.6, -8.6, -8.5, -8.2, -8.0, -7.9, -7.9, -7.9, -7.8, -7.8, -7.6, -7.6, -7.6, -7.6, -7.6, -7.6, -7.6 and -7.5kcal/mole respectively. Many of the phytochemicals (having energy ≤7.5 kcal/mole) were also predicted to interact with SARS-CoV-1 nucleocapsid protein (PDB ID: 2cjr) viz, Ursolic acid, Somniferine A, Oleanolic acid, Magnoflorine, Withanolide B, Withanolide, Withanone, Orientin, Withaferin A, Quercetin, Rhamnetin and Isothymusina. Their predicted binding energies are -9.0, -8.6, -8.4, -8.2, -8.2, -7.9, -7.8, -7.7, -7.7, -7.6, -7.6 and -7.5 kcal/moles respectively.

Table 7 shows the predicted binding energy of different phytochemicals with molecules involved in the inflammatory processes such as COX2, PLA2, NIK and IRAK-4. From Table 7, it is clear that Withaferin A, Withanolide B, Withanolide, Withanone, Campesterol, Cyclocurcumin,Somniferine A, Stigmasterol, Eriodictyol, Isopiperine, Oleanolic acid, Rhamnetin, Orientin, Quercetin, Piperine and Vicenin may possess high binding affinity with most of the inflammatory molecules used in the study. Our study proposes that many of the phytochemicals present in these herbs may directly inhibit COX-2, PLA2 and IRAK-4 which are involved in the inflammation. Many of these phytochemicals may also bind to the active site of the protein and at the same time few of them may have very high affinity for other regions of the target protein which may affect its function. Our study also shows that several phytochemicals present in the *Kadha* have significant predicted inhibitory activity towards NIK. Inhibition of the NIK by phytochemicals may moderate the NF-κB dependent genes which are involved in inflammation. Based on these docking studies, it may be predicted that the phytochemicals present in the *Kadha* may exhibit anti-inflammatory property.

Discussion

Use of herbs and phytochemicals has a long history in the management of various respiratory diseases (Santana et al., 2016; Alamgeer et al., 2018; Pinn et al., 2001). Currently, the demand of complementary medicine, including herbal medicine has become more popular in healthcare for both general maintenance of health and for treatment of minor illnesses (Barnes, 2004). In European countries, several species of herbs have been used against flu and common cold (Weiss and Fintelmann, 2000). Similarly, in Russia and Estonia various herbs and medicinal plants have also been used for centuries for management of common cold and flu (Raal et al., 2013). In India, use of spices and herbs for treatment of various diseases including cough, cold is a common practice with recorded history of over 2000 years (Vasanthi and Parameswari, 2010, Sachan et al., 2018).

It is well-known that SARS-CoV viral genome encodes more than 20 proteins, among which two proteases i.e 3-chymotrypsin-like protease (3CLpro, main protease, Mpro) and papain-like protease (PLpro) are vital for virus replication (Lindner et al. 2005). They cleave the two translated polyproteins (PP1A and PP1AB) into individual functional components, resulting in release of 16 non-structural proteins (NSPs) (Jo et al., 2020). Thus SARS-CoV-2 main protease is considered as a promising druggable target. The viral NSPs play an important role in replication and transcription. Our study predicts that many of the phytochemicals of *Kadha* have significant binding affinity with the main protease. Twelve phytochemicals namely Somniferine A, Tinosporide, Tinocordioside, Orientin, Flavonol glucoside, Withanolide, Apigenin, Cyclocurcumin, Withanolide B, Kaempferol, Withanone and Withaferin A, have predicted binding energy lower than the pharmacological inhibitor, N3 (Fig 1, Table 2). The binding of these phytochemicals with main protease may slow down the cleavage of PPs to releases NSPs and decrease the process of viral replication and transcription.

The SARS-CoV spike protein plays an important role in virus entry into the host (Li, 2016). Initial interactions between the S1 domain and its host receptor (ACE2), and subsequent S2 segment mediated fusion of the host and viral membrane allows the viral RNA genome to enter inside the host cells. Thus, these proteins represent as important targets for designing drug (Li, 2016). Our study predicts that an array of the phytochemicals have significant binding affinity with the SARS-CoV-2 spike proteins (Table 3, Figure 2a) as well as with host ACE2

protein (Table 4, Figure 2b) and furin protein (which facilitate spike and ACE2 interaction) (Table 5, Figure 3). Thus, phytochemicals may significantly inhibit viral interaction with the host receptor and slow down or stop the entry of the viral genome inside the host. The spike protein is also known to activate the immune response of the host cell towards CoVs (Li, 2016). The S1 domain of spike acts as a major antigen on the surface of the virus (Yuan et al., 2016).

SARS-CoV nucleocapsid protein (SARS-CoV NP) is another vital structural protein which shows intrinsic multimerization and interacts with M protein, suggesting that NP is both critical to formation of the viral nucleocapsid core and is involved in virion assembly (He et al. 2004, He et al. 2004). Our study predicts that the phytochemicals such as Withanone, Withanolide B, Withanolide, Withaferin A, Ursolic acid, Tinosporaside, Tinocordioside, Stigmasterol, Somniferine A, Quercetin, Oleanolic acid and Crategolic acid have significant binding energy with nucleocapsid protein (Table 6). The N protein of SARS-CoV are also known to up-regulate the expression of the proinflammatory protein COX2 and also interact with the proteasome subunit p42, which affects a variety of basic cellular processes and inflammatory responses (Hu et al. 2017; Wang et al. 2010).

Nsp3 protein is another key component for Coronavirus and is essential for replication/transcription complex (RTC) formation. It plays various roles in Coronavirus infection. It releases Nsp1, Nsp2, and itself from the polyproteins and interacts with other viral Nsps as well as RNA to form the replication/ transcription complex (Leia et al. 2018). Similarly, Nsp15 protein also acts as an endoribonuclease and preferentially cleaves 3' of uridylates through a ribonuclease A (RNase A)-like mechanism and also facilitates viral replication and transcription (Alcantara et al. 2010; Sawicki and Sawicki, 1998). Our study predicted that many of the phytochemicals of the *Kadha* have significant binding affinity with Nsp3 and Nsp15. Thus, the phytochemicals identified in Table 6 may disrupt the formation of RTC and stop the viral genome replication (Table 6).

During the SARS-CoV infection, human lung epithelial cells are among the first targets for viral entry. In response to viral multiplication and host cell damage, lung epithelial cells secrete inflammatory mediators to initiate and exacerbate host innate inflammatory responses, causing detrimental immune-mediated pathology within the lungs. SARS-CoV-2 infects ACE-2 expressing epithelial cells in the air sacs (alveoli) in lower lungs. The damaged epithelium leads

and interlobular septal thickening lead to leaky cell junctions, accumulation of fluid which is rich in proteins, inflammatory mediators and Pneumonia. The spread of virus from lung to systemic circulation and excessive production of pro-inflammatory mediator can damage different vital organs. Our study predicts that an array of the phytochemicals such as Withaferin A, Withanolide B, Withanolide, Withanone, Campesterol, Cyclocurcumin,Somniferine A,Stigmasterol, Eriodictyol, Isopiperine, Oleanolic acid, Rhamnetin, Orientin, Quercetin, Piperine, Vicenin etc. found in the preparation of the *Kadha,* have significant binding affinity with the many of these inflammatory mediators or the molecules involved in this process (Table 7). It is well known that, NF-κB is the master regulator for several genes such as COX-2, VEGF (vascular endothelial growth Factor), pro-inflammatory cytokines (IL-1, IL-2, IL-6, and TNFα), chemokines (e.g., IL-8, MIP-1α, and MCP-1), adhesion molecules, immunoreceptors, growth factors, and other agents involved in proliferation and invasion. NF-κB activation is mediated by two distinctly different redox-related signalling pathways. The first pathway involves NIK/IKK whereas second pathway involve MAPKs and both causes the induction of transcriptional activation of NF-κB. NF-κB-inducing kinase (NIK) is a key mediator of the non-canonical NF-κB signalling pathway. NF-κB is also one of the main inducible transcription factors shown to respond directly to oxidative stress. Oxidative stress activates nuclear factor-inducing kinase (NIK)/IκB kinase (IKK) and mitogen-activated protein kinases (MAPKs). NIK/IKK and MAPK pathways activate NF-κB and migrates to the nucleus and binds to κ elements on DNA in enhancers and promoter regions. Various herbs have potential to control inflammation-associated disease by decreasing the production of the pro-inflammatory mediators by suppressing pro-inflammatory pathways (Pan et al., 2011). Our study also predicted that phytochemicals found in the *Kadha* have significant binding affinity with NIK (Table 7) which can stop NF-κB mediated downstream events. In a very recent study, Huang et al (2020) have shown that the patients infected with SARS-CoV-2 had high amounts of IL1β, IFNγ, IP10, and MCP1, which can mediate cytokine storm associated multi-organ damage. At the same time, SARS-CoV-2 infection also initiates increased secretion of T-helper-2 (Th2) cytokines (eg, IL4 and IL10) which suppress inflammation (Huang et al., 2020). The increased secretion of inflammatory mediators was also associated with moderation of helper T cell responses in COVID19 patients.

Taken together, our current findings and the recent knowledge about SARS-CoV and SARS-CoV-2 pathology, profess the use of Ayurvedic *Kadha* in the prevention and management of COVID-19. The phytochemicals found in the *Kadha* have significant binding affinity with the different CoVs proteins (Scheme 1), indicating that they may control viral infection and multiplication in the host cells. Molecular docking study with human inflammatory mediators predicts that many of the phytochemicals present in this preparation have significant anti-inflammatory property. Most of the phytochemicals found in the herbs ashwagandha, giloy, tulsi, clove and black pepper have potential to interact with most of the druggable proteins selected in this study. In conclusion, regular consumption of ayurvedic *Kadha* in consultation with ayurvedic practitioner may decrease the inflammatory response, boost the individual's immunity and reduce the risk of CoVs infection including SARS-CoV-2.

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Legends

Figure 1: SARS-CoV-2 main protease (PDB ID: 6LU7) showing top 12 phytochemicals superimposed on it active site.

Figure 2: Selected phytochemicals superimposed on SARS-CoV-2 spike and human ACE2. a) SARS-CoV-2 spike, b) Human ACE2.

Figure 3: Selected phytochemicals superimposed on human furin (PDB ID: 5MIM).

Scheme 1: Possible targets for the phytochemicals found in the *Kadha* against different SARS-CoV-2 proteins.

Table 1: The predicted binding of different phytochemicals against SARS-CoV-2 main protease, spike protein, human ACE2 and furin proteins. Only those phytochemicals name are included in the table which have binding energy ≤ 7.5 kcal/mole.

Table 2: The predicted binding and 2D interaction of top 12 phytochemicals against SARS-CoV-2 main protease (PDB ID: 6LU7) and their different protein residues which are involved in the interaction.

Table 3: The predicted binding and 2D interaction of top 12 phytochemicals against SARS-CoV-2 spike protein (PDB ID: 6VXX) and their different protein residues which are involved in the interaction.

Table 4: The predicted binding and 2D interaction of top 10 phytochemicals against human ACE2 protein (PDB ID: 2AJF) and their different protein residues which are involved in the interaction.

Table 5: The predicted binding and 2D interaction of top 12 phytochemicals against human furin protein (PDB ID: 5MIM) and their different protein residues which are involved in the interaction.

Table 6: The predicted binding of different phytochemicals against SARS-CoV-2 and SARS-CoV target proteins. Only those phytochemicals name are included in the table which have binding energy ≤ 7.5 kcal/mole.

Table 7: The predicted binding of different phytochemicals against different anti-inflammatory macromolecules. Only those phytochemicals name are included in the table which have binding energy ≤ 7.5 kcal/mole.

Figure 1: SARS-CoV-2 main protease (PDB ID: 6LU7) showing top 12 phytochemicals superimposed on it active site.

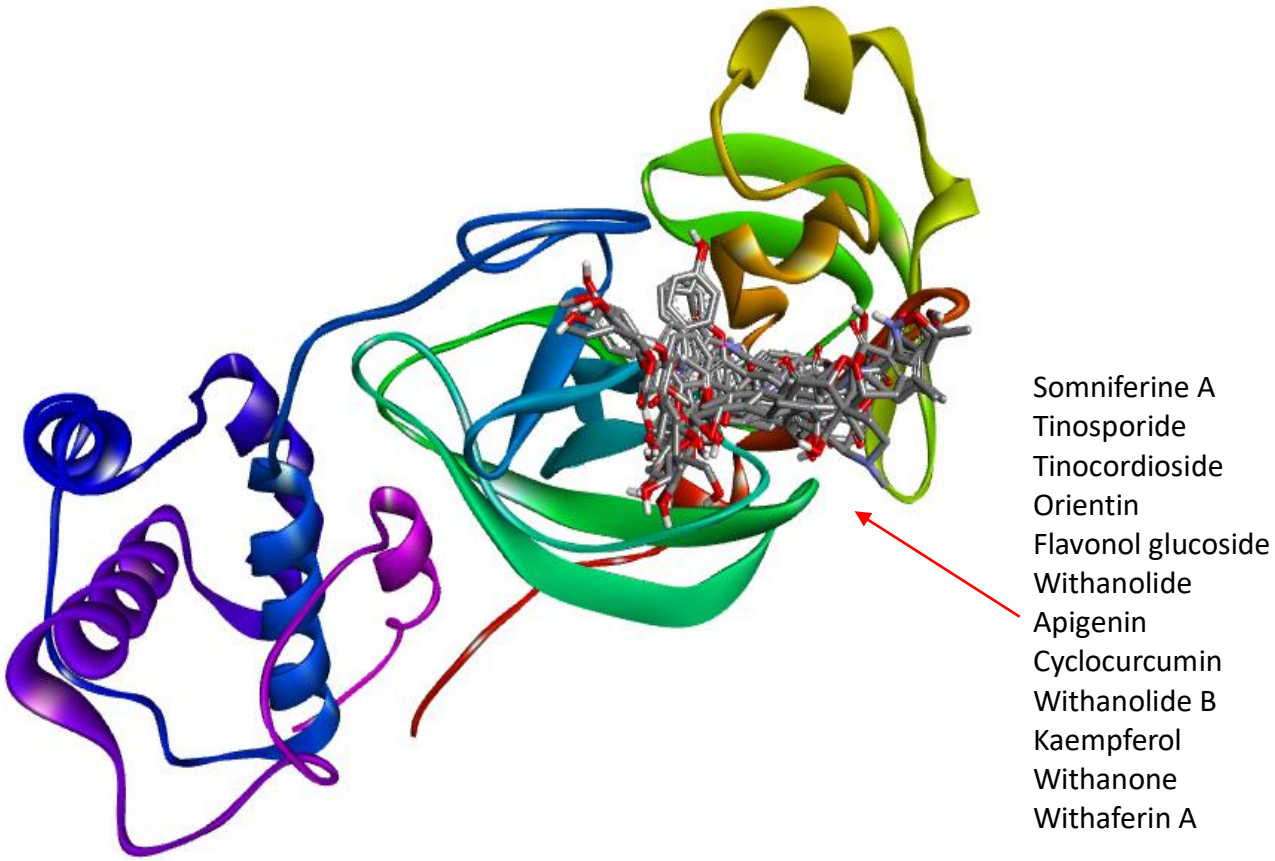
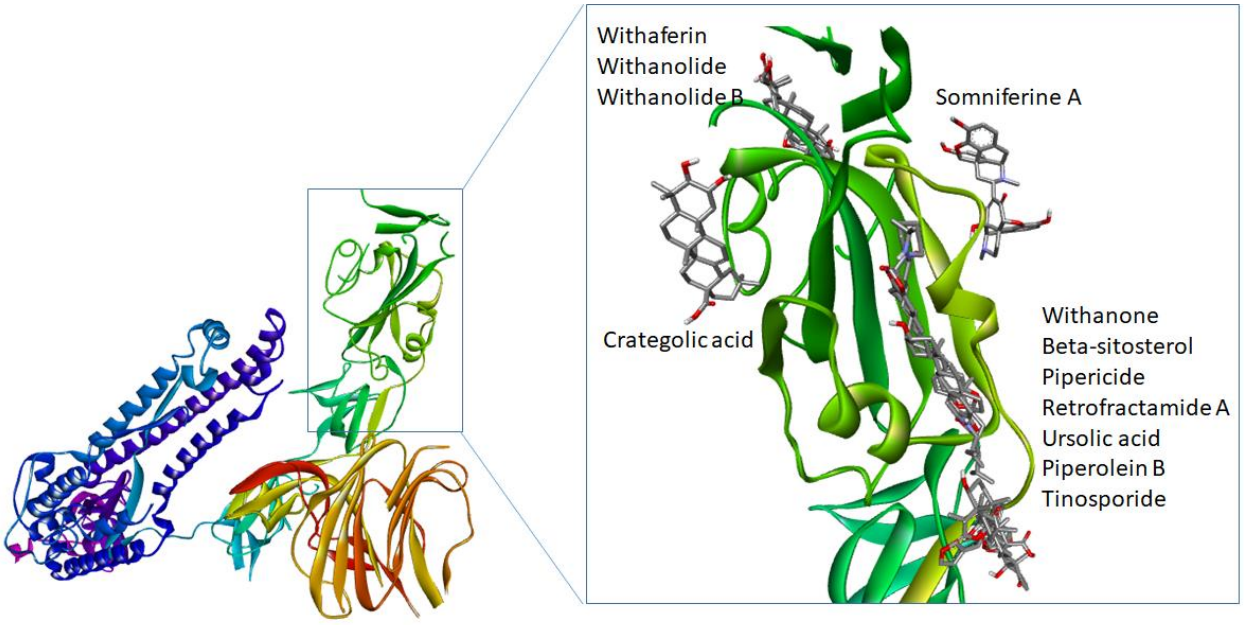


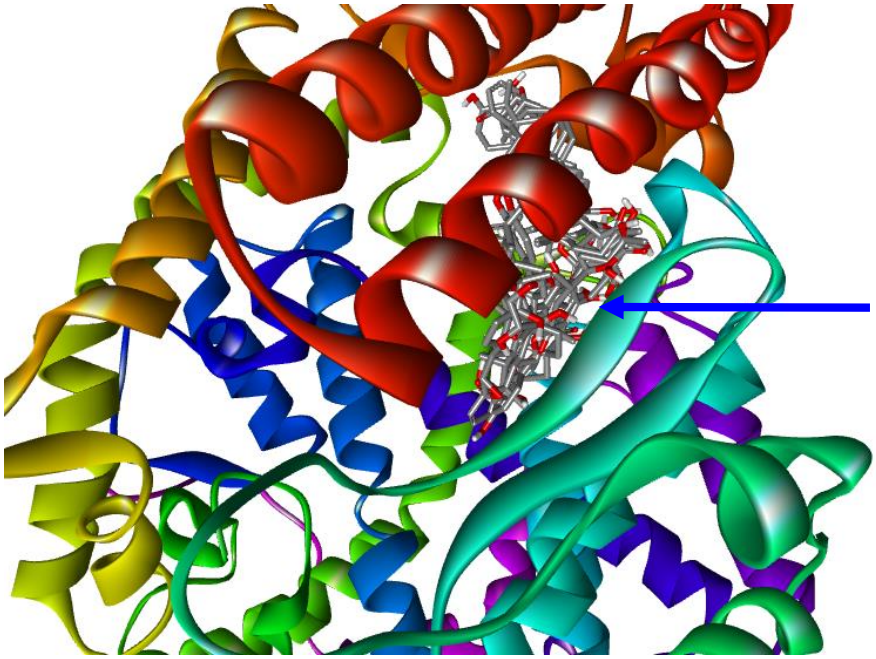
Figure 2: Selected phytochemicals superimposed on SARS-CoV-2 spike and human ACE2. a) SARS-CoV-2 spike, b) Human ACE2.

a.



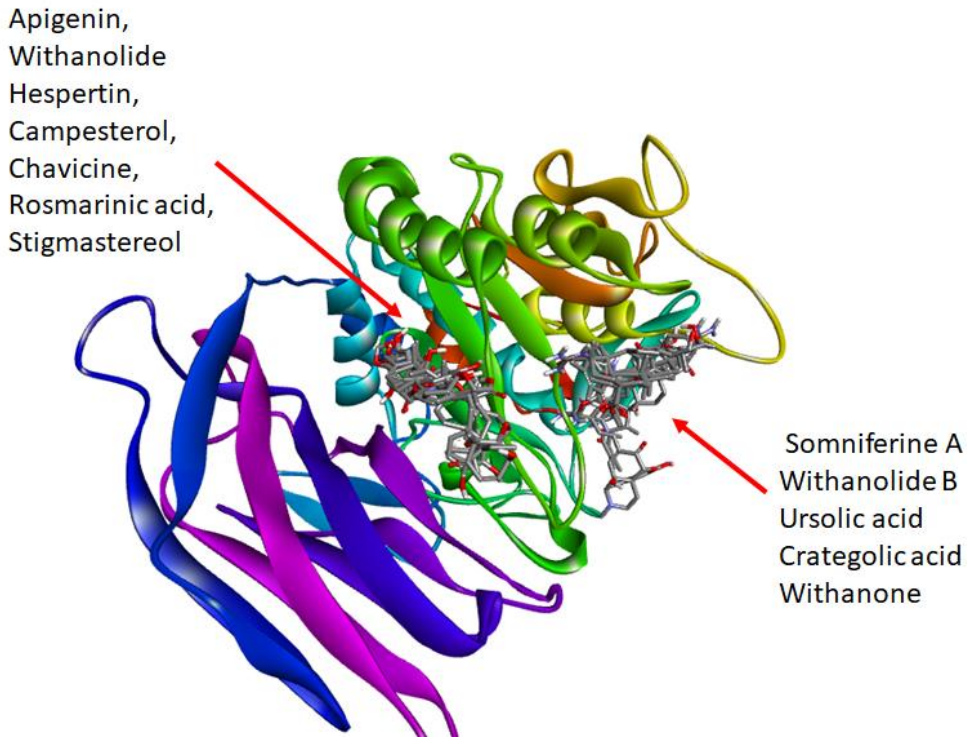
SARS-CoV-2 spike protein which interact with ACE2

b.



Withanolide
Somniferine A
Apigenin
Withanolide B
Ursolic acid
Hesperetin
Campesterol
Crategolic acid
Withanone
Chavicine
Rosmarinic acid
Stigmasterol.

Figure 3: Selected phytochemicals superimposed on human furin (PDB ID: 5MIM).



Scheme 1: Possible targets for the phytochemicals found in the *Kadha* against different SARS-CoV-2 proteins.

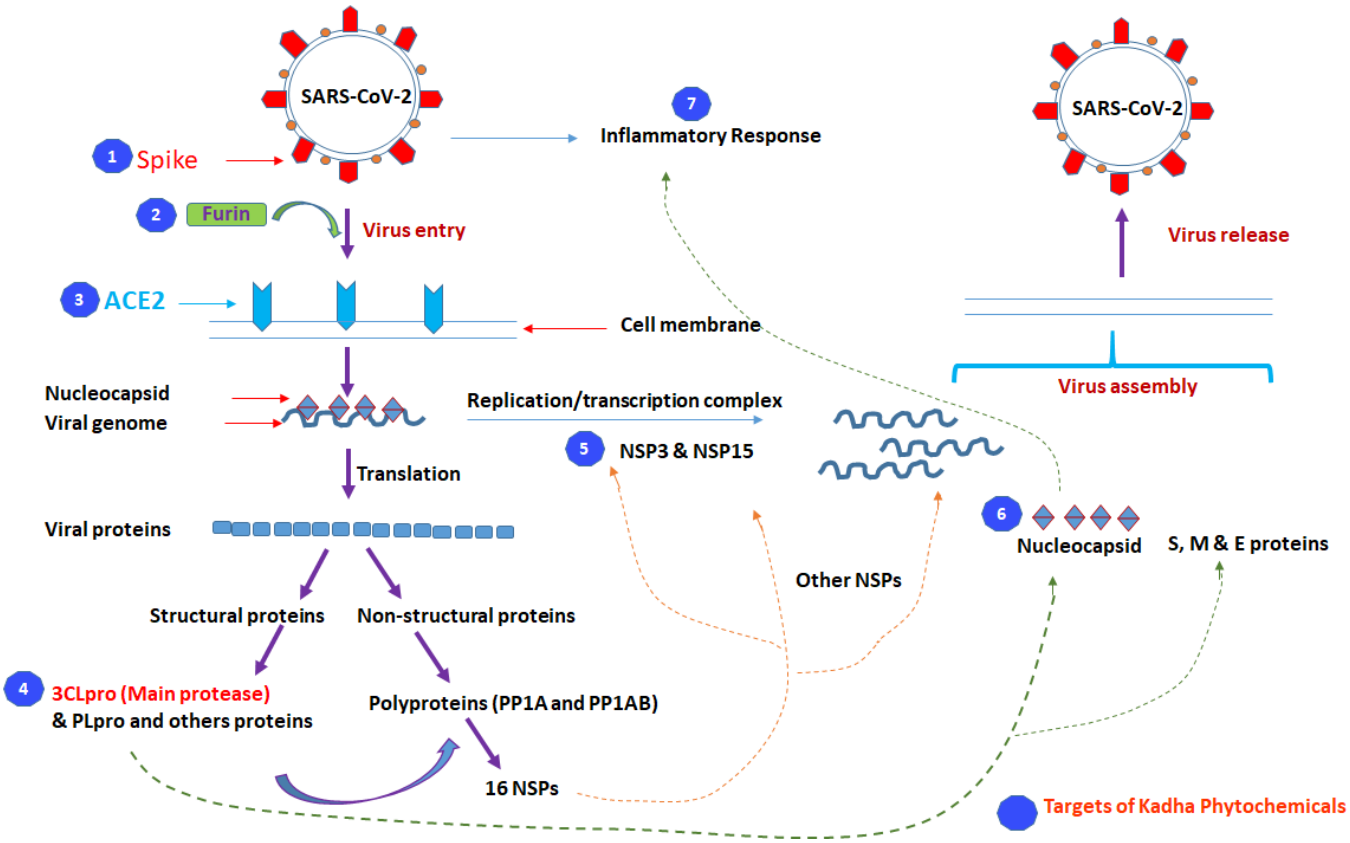
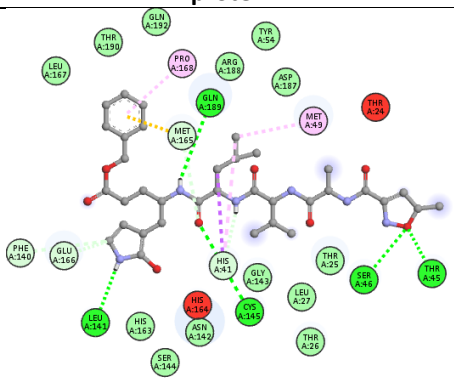
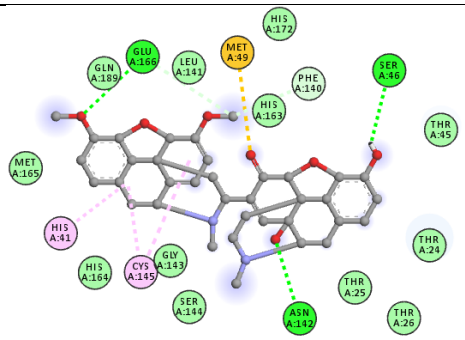
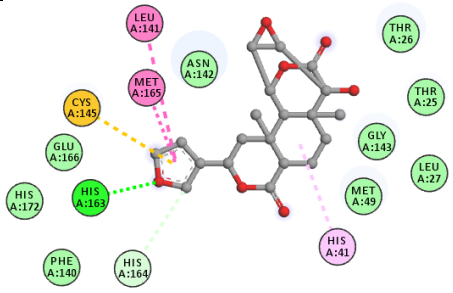
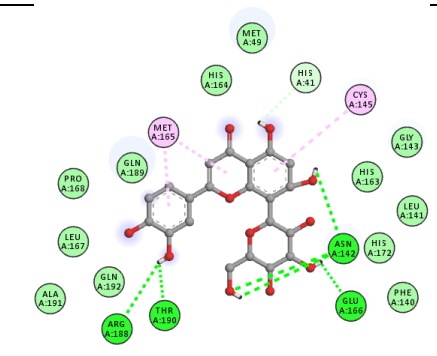


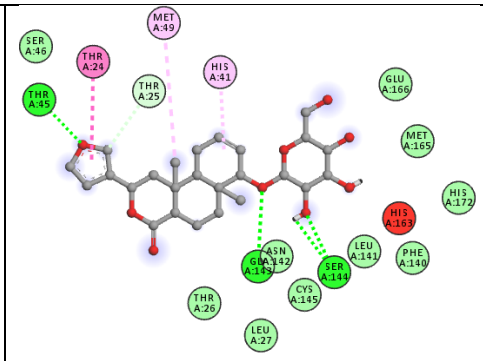
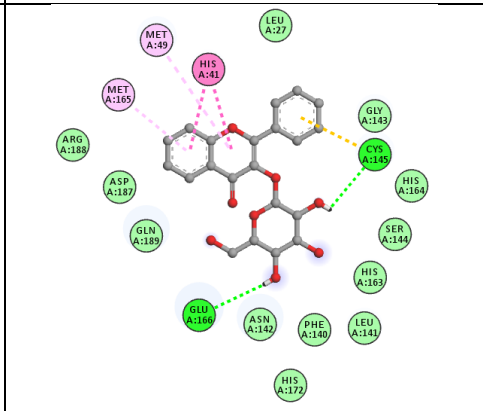
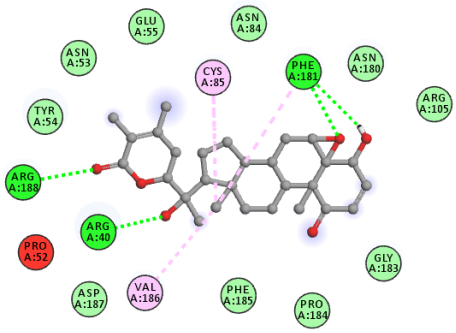
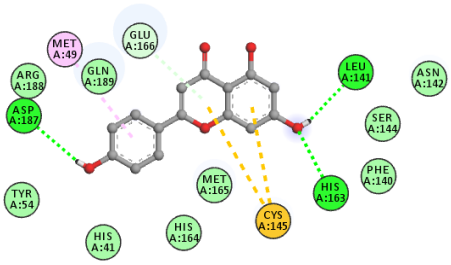
Table 1

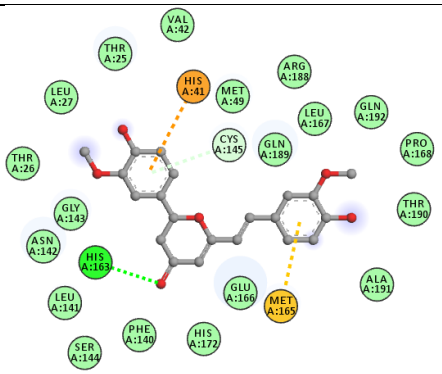
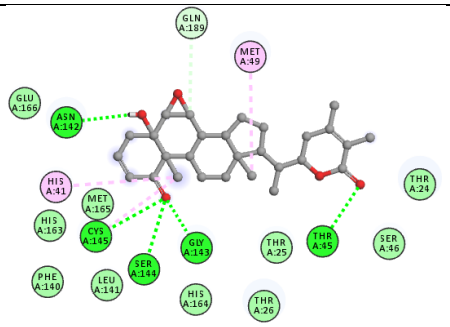
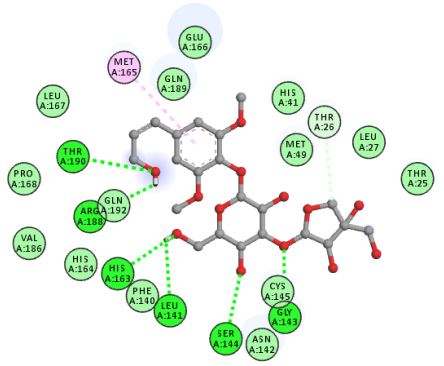
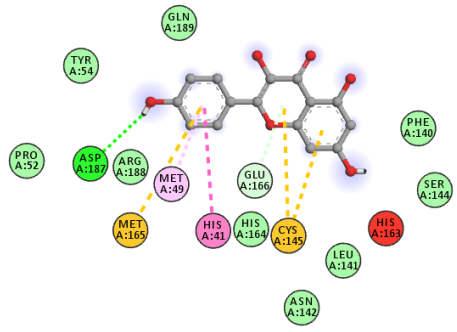
Compounds	Target molecules; binding energy (kcal/mole) (Only those phytochemcals are included in the table which have binding energy ≤ -7.5)			
	COVID-19 main protease (PDB ID: 6lu7)	**SARS-CoV-2 spike protein (PDB ID: 6vxx)**	**Human ACE2 (PDB ID: 2ajf)**	**Human Furin (PDB ID: 5mim)**
Native ligand (N3)	-7.6			-9.3
Phytochemicals				
Apigenin	-7.8		-7.5	-8.5
Berberine			-7.5	-7.8
Beta-sitosterol		-7.6	-8.3	-7.6
Bisdemethoxycurcumin			-8.1	-7.7
Brachyamide B			-7.7	-7.7
Campesterol			-8.3	-0.1
Chavicine				-8
Cordifolioside A	-7.7		-7.5	
Cordioside			-7.8	
Crategolic acid	-7.6	-7.5	-8.6	-8.1
Curcumin	-7.8			-7.8
Cyclocurcumin			-8.3	
Demethoxycurcumin			-7.6	
Eriodictyol			-7.7	
Flavonol glucoside	-8		-7.5	
Hesperetin			-7.7	-8.2
Isochavicine			-7.8	-7.5
Isopiperine				-7.6
Isothymonin			-7.6	
Jatrorrhizine				-7.5
Kaempferol	-7.7		-7.9	
Oleanolic acid			-8.5	-7.9
Orientin	-8.1		-8	-7.9
Piperettine			-8	-7.8
Pipericide		-7.6	-7.5	
Piperolein b			-7.6	
Quercetin			-8	
Retrofractamide A		-7.5		
Rhamnetin			-7.5	
Rosmarinic acid				-8
Somniferine A	-8.6	-7.8	-9	-8.7
Stigmasterol			-8.8	-8.5
Tinocordifolioside	-7.6			
Tinocordioside	-8.1		-8.5	-8
Tinosporaside			-8.1	-8
Tinosporide	-8.6		-8.3	-7.6

Trichostachine				-7.8
Ursolic acid	-7.7	-7.5	-8.7	-8.3
Vicenin			-8.8	-7.7
Withaferin A	-7.6	-8.2	-9.1	-7.9
Withanolide	-8	-8.4	-9.2	
Withanolide B	-7.8	-8.7	-8.7	-8.4
Withanone	-7.6	-7.9	-8.5	-8.1

Table 2: 2D interaction of top 12 phytochemicals with SARS-CoV-2 main protease (PDB ID: 6LU7)

S. No.	Name of the molecules	2D interaction between molecules and protein	Kinds of the binding
1	Native ligand N3 Binding energy= -7.6		**Convention hydrogen bonding:** THR45, SER46, LEU141, CYS145, GLN189 **Van der vaals:** THR25, THR26, LEU27, HIS41, PHE140, ASN142, GLY143, SER144, HIS163, MET165, GLU166, LEU167,ASP187, ARG188, THR190, GLN192 **Pi-alkyl:** MET49, PRO168 **Pi-Sigma:** HIS41 **Pi-Sulphur:** PRO168
2	Somniferine A Binding energy= -8.6		**Convention hydrogen bonding:** SER46, ASN142, GLU166 **Van der vaals:** THR24, THR25, THR26, THR45, GLY143, SER144, HIS163, HIS164, MET165, **Pi-alkyl:** HIS41, CYS145 **Pi-sulphur:** MET49 **Carbon Hydrogen bond:** PHE140, LEU141
3	Tinosporide Binding energy=-8.6		**Convention hydrogen bonding:** HIS163 **Van der vaals:** THR25, THR26, LEU27, MET49, PHE140, ASN142, GLY143, HIS164, GLU166, HIS172 **Pi-alkyl:** HIS41 **Amide –Pi stacked:** EU141, MET165 **Pi-Sulphur:** CYS145
4	Orientin Binding energy=-8.1		**Convention hydrogen bonding:** ASN142, GLU166, ARG188, THR190 **Van der vaals:** HIS41, MET49, PHE140, LEU142, GLY143, HIS163, HIS164,LEU167, PRO168, GLN189, ALA191, GLN192, HIS172,ALA191, GLN192 **Pi-alkyl:** CYS145, MET165

5	Tinocordioside Binding energy=-8.1		**Convention hydrogen bonding:** THR45, GLA143, SER144 **Van der vaals:** THR25, THR26, LEU27,SER46, PHE140, LEU141, ASN142, CYS145, MET165, GLU166, HIS172 **Pi-alkyl:** HIS41, MET49 **Pi-amide:** THR24
6	Flavonol glucoside Binding energy=-8.0		**Convention hydrogen bonding:** CYS145, GLU166 **Van der vaals:** LEU27, PHE140, LEU141, ASN142, GLY143, SER144, HIS163, HIS164, HIS172, ASP187, ARG188, GLN189 **Pi-alkyl:** MET49, MET165 **Pi-Sulphur:** CYS145
7	Withanolide Binding energy=-8.0		**Convention hydrogen bonding:** ARG40, PHE181, ARG188 **Van der vaals:** ASN53, TYR54, GLU55, ASN88, ARG105, ASN180, GLY183, PRO184, PHE185 **Pi-alkyl:** CYS85, PHE181, VAL186
8	Apigenin Binding energy=-7.8		**Convention hydrogen bonding:** LEU141, HIS163, ASP187 **Pi donor hydrogen bond:** GLU166 **Van der vaals:** HIS41, TYR154, PHE140, ASN142, SER144, HIS164, MET165, GLN89, ARG188, **Pi-alkyl:** MET49 **Pi-Sulphur:** CYS145

9	Cyclocurcumin Binding energy=-7.8		**Convention hydrogen bonding:** HIS163 **Van der vaals:** THR25, THR26, LEU27, VAL42, MET49, PHE140, LEU142, ASN142, GYL143, SER144, GLU166, LEU167, HIS172, ARG188, GLN189, THR190, ALA91, GLN192, **Pi-cation:**HIS41 **Pi-sulphur:** MET65 **Pi-Donor hydrogen bond:** CYS145
10	Withanolide B Binding energy=-7.8		**Convention hydrogen bonding:** THR45, ASN42, GLY43, SER144, CYS145 **Van der vaals:** THR24, THR25, THR26, SER46,PHE140, LEU142, HIS164, MET165, GLU166 **Pi-alkyl:** HIS41, MET49 **Carbon-Hydrogen bond:** GLN189
11	Cordifolioside A Binding energy=-7.7		**Convention hydrogen bonding:** LEU142, GLY143, SER144, HIS163, ARG188, THR190 **Van der vaals:** THR25, LEU27, MET49, PHE140, ASN142, CYS145, HIS164, GLU166, PRO168, LEU167, VAL186, GLN189, GLN192 **Pi-alkyl:** MET165 **Carbon-Hydrogen bond:** THR26
12	Kaempferol Binding energy=-7.7		**Convention hydrogen bonding:** ASP187 **Van der vaals:** PRO52, TYR54, PHE140, LEU141, ASN142, SER144, HIS164, ARG188, GLN189 **Pi-alkyl:** MET49 **Pi-Sulphur:** CYS145, MET165 **Pi-Donor hydrogen bond:** GLU166 **Pi-Pi stacked:** HIS41

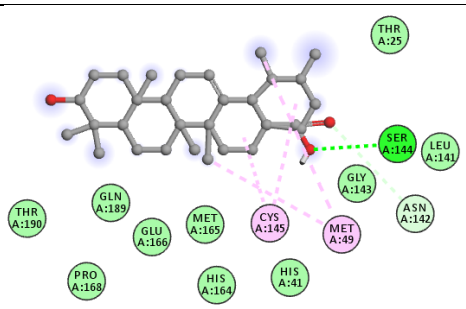
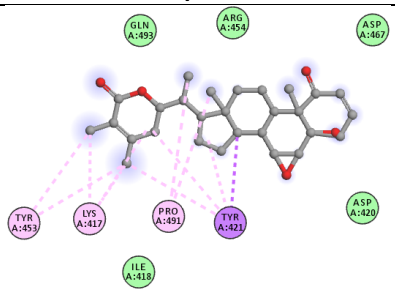
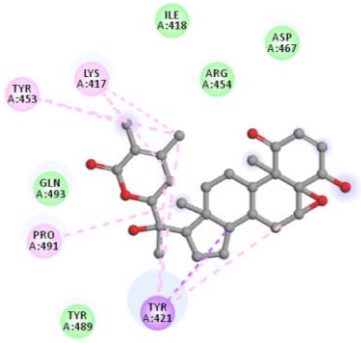
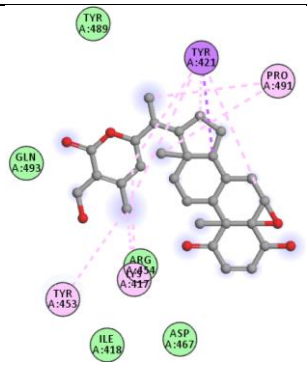
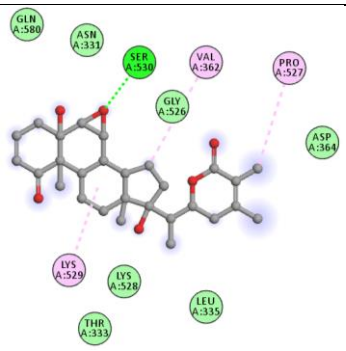
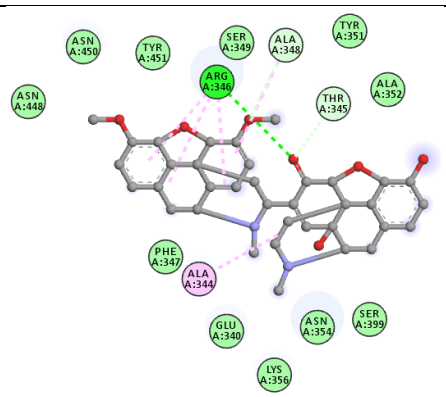
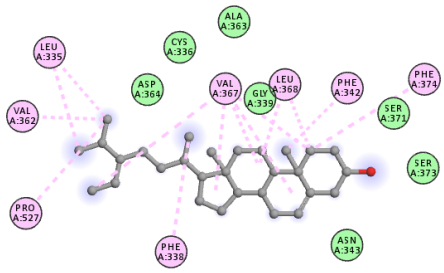
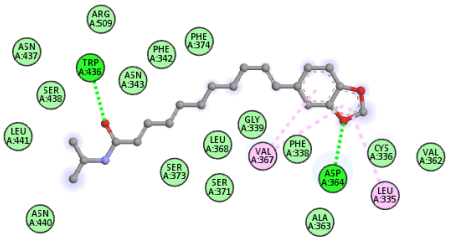
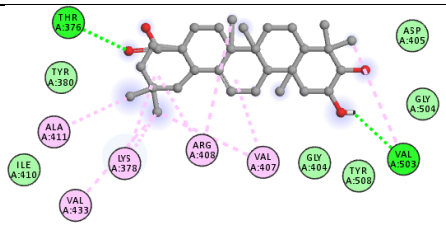
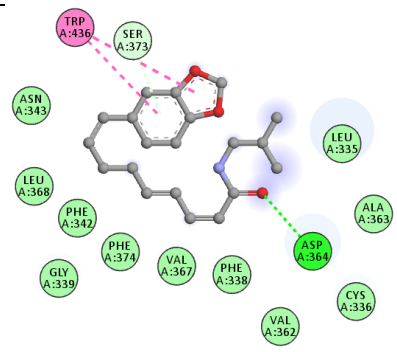
13	Ursolic acid Binding energy=-7.7		**Convention hydrogen bonding:** SER144 **Van der vaals:** THR25, HIS41, LEU141, GLY143, HIS164, MET165, GLU166, PRO168, GLN189, HR190 **Pi-alkyl:** MET49, CYS145 **Carbon-Hydrogen donor:** ASN142

Table 3: 2D interaction of top 12 phytochemicals with SARS-CoV-2 Spike (PDB ID: 6VXX)

S. No.	Name of the molecules	2D interaction between molecules and protein	Kinds of the binding
1	Withanolide B Binding energy=-8.7		**Van der vaals:** ILE418, ASP420, ARG454, ASP467, GLN493 **Pi-alkyl:** LYS417, TYR453, PRO491 **Pi-Sigma:**TYR421
2	Withanolide Binding energy=-8.4		**Van der vaals:** ILE418, ARG454, ASP467, TYR489, GLN493 **Pi-alkyl:** LYS417, TYR453, PRO491 **Pi-Sigma:** TYR421
3	Withaferin A Binding energy=-8.2		**Van der vaals:** ILE418, ARG454, ASP467, TYR489, GLN493 **Pi-alkyl:**CYS417, TYR453, PRO491 **Pi-Sigma:** TYR421
4	Withanone Binding energy=-7.9		**Convention hydrogen bonding:** SER530 **Van der vaals:** ASN331, THR333, LEU335, ASP364, GLY526, LYS528, GLN580 **Pi-alkyl:** VAL362, PRO527, LYS529

5	Somniferine A Binding energy=-7.8		**Convention hydrogen bonding:** ARG346 **Van der vaals:** GLU340,THR345, PHE347, ALA348, SER349, ASN354, LYS356, SER399, ASN448, ASN450, TYR451 **Pi-alkyl:** ALA344
6	Beta-sitosterol Binding energy=-7.6		**Van der vaals:**CYS336, GLY339, ASN343, ALA363, ASP364, SER371, SER373 **Pi-alkyl:** LEU335, PHE338, PHE342, VAL362, VAL367, LEU368, PHE374, PRO527
7	Pipericide Binding energy=-7.6		**Convention hydrogen bonding:** ASP364, TRP436 **Van der vaals:** CYS336, PHE338, GLY339, PHE342, ASN343, VAL362, ALA363, LEU368, SER371, SER373, PHE374, ASN437, SER438, ASN440, LEU441, ARG509 **Pi-alkyl:** LEU335, VAL367
8	Crategolic acid Binding energy=-7.5		**Convention hydrogen bonding:** THR376, VAL503 **Van der vaals:** TYR380, GLY404, ASP405,ILE410, GLUY504, TYR508 **Pi-alkyl:** LYS378, VAL407, ARG408, ALA411, VAL433
9	Retrofractamide A Binding energy=-7.5		**Convention hydrogen bonding:** ASP364 **Van der vaals:** LEU335, CYS336, PHE338, GLY339, ASN343, PHE342, VAL367, LEU368, SER373 **Pi-Pi:** TRP436

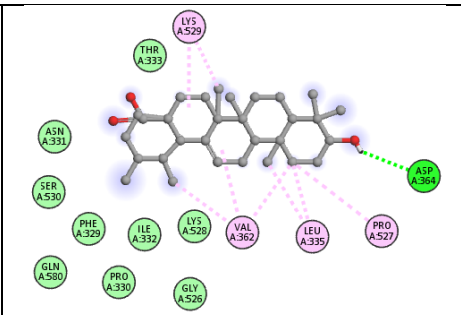
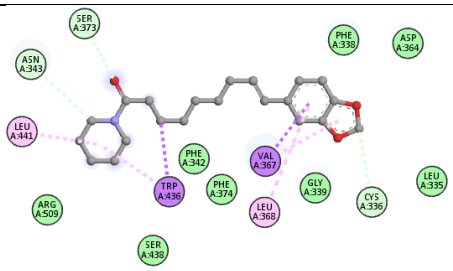
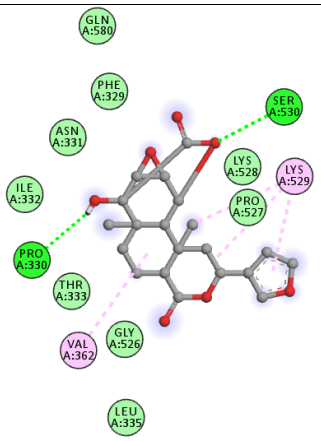
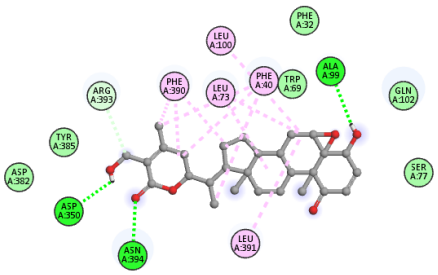
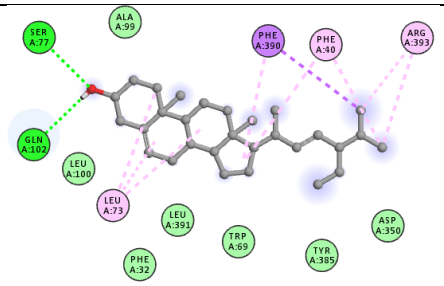
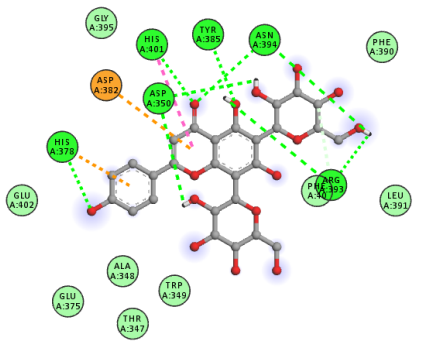
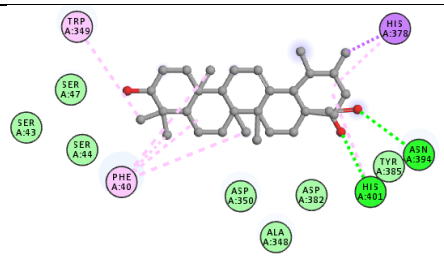
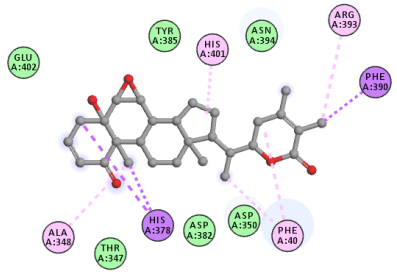
10	Ursolic acid Binding energy=-7.5		**Convention hydrogen bonding:** ASP364 **Van der vaals:** PHE329, PRO330, ASN331, ILE332, THR333, GLY526, LYS528, SER530, GLN580, **Pi-alkyl:** LEU335, VAL362, PRO527, LYS529
11	Piperolein B Binding energy=-7.4		**Van der vaals:** LEU335, PHE338, GLY339, PHE342, ASP364, PHE374,SER438, ARG509 **Carbon hydrogen bond:** CYS336, ASN343, SER373 **Pi-alkyl:** LEU368, LEU441 **Pi-Sigma:** VAL367, TRP436
12	Tinosporide Binding energy=-7.4		**Convention hydrogen bonding:** PRO330, SER530 **Van der vaals:** PHE329, ASN331, ILE332, THR333, LEU335, GLY526, PRO527, LYS528, GLN580 **Pi-alkyl:** VAL362, LYS529

Table 4: 2D interaction of top 10 phytochemicals with human ACE2 (PDB ID: 2AJF)

S. No.	Name of the molecules	2D interaction between molecules and protein	Kinds of the binding
1	Withaferin A Binding Energy:-9.1		**Convention hydrogen bonding:** AL99, ASP350, ASN394 **Van der vaals:** PHE32, SER77, GLN102, ASP382, TYR385, ARG393 **Pi-alkyl:** PHE40, LEU73, LEU100, PH1390, PHE391
2	Stigmasterol Binding Energy:-8.8		**Convention hydrogen bonding:** SER77, GLN102 **Van der vaals:** PHE32, TRP69, ALA99, LEU100, ASP350, TYR385, LEU391 **Pi-alkyl:** PHE40, LEU73, ARG393 **Pi-Sigma:** PHE390
3	Vicenin Binding Energy:-8.8		**Convention hydrogen bonding:** ASP350, HIS378, TYR385, ARG393, ASN394, HIS401 **Van der vaals:** PHE40, THR347, ALA348, TRP349, GLU375, PHE390, LEU391, GLY395, GLU402 **Pi-Pi stack:** HIS401 **Pi-cation:** ASP382
4	Ursolic acid Binding Energy:-8.7		**Convention hydrogen bonding:** ASN394, HIS01 **Van der vaals:** SER43, SER44, SER47, ALA348, ASP350, ASP382, TYR385 **Pi-alkyl:** PHE40, TRP349, HIS378 **Pi-Sigma:** HIS378
5	Withanolide B Binding Energy:-8.7		**Pi-alkyl:** PHE40, ALA348, ARG393, HIS401 **Van der vaals:** THR347, ASP350, ASP382, TYR385, ASN394, GLU402 **Pi-Sigma:** HIS378, PHE390

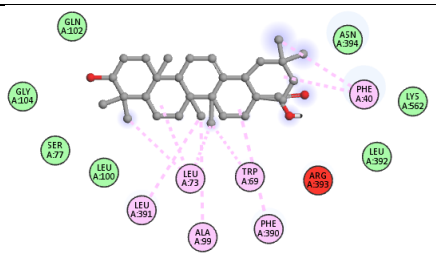
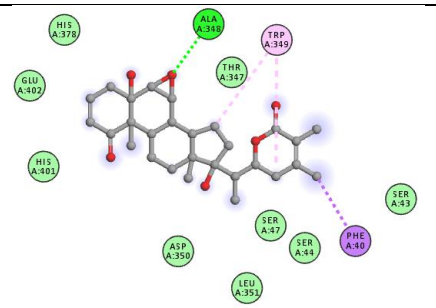
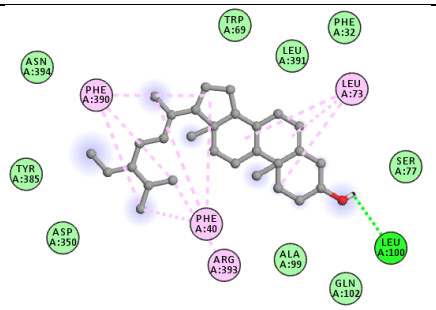
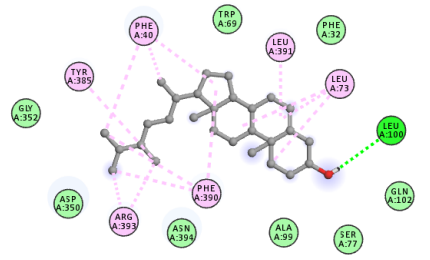
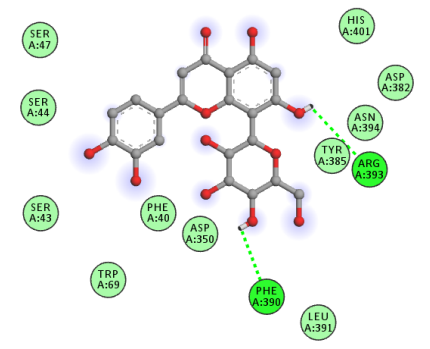
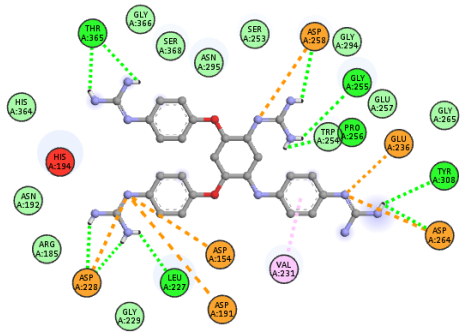
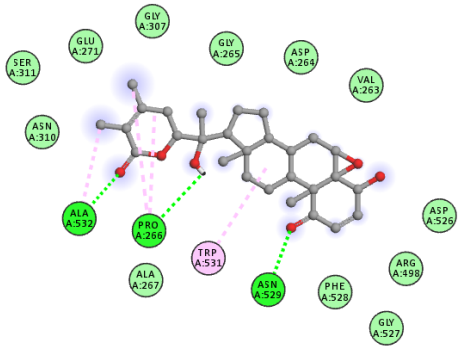
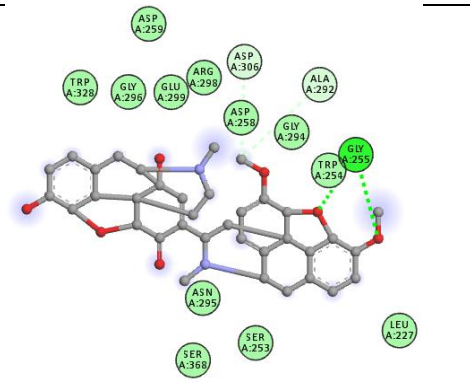
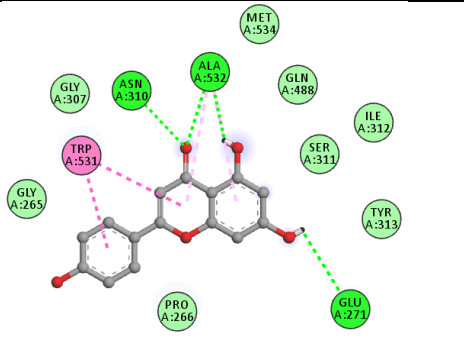
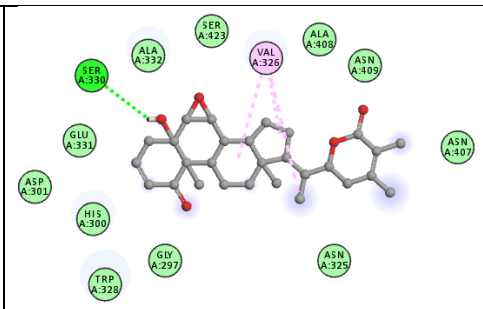
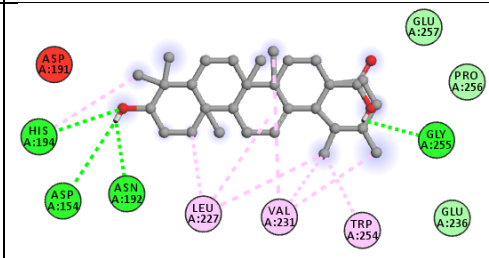
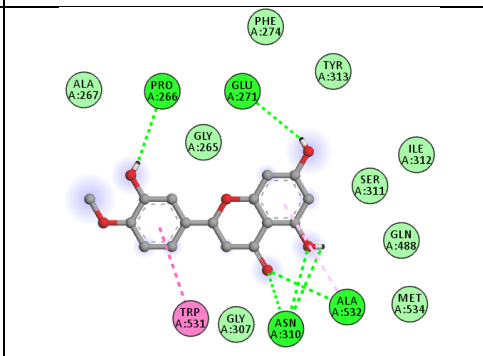
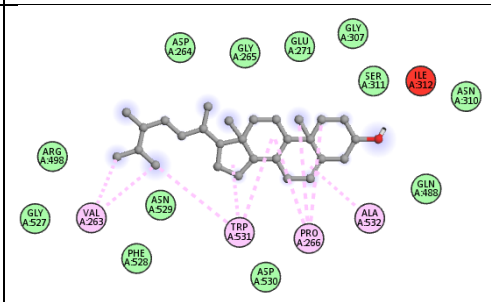
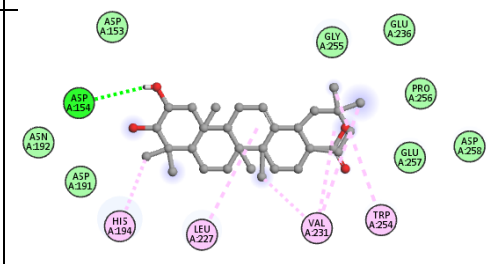
6	Oleanolic acid Binding Energy:-8.5		**Van der vaals:** SER77, LEU100,GLN102,GLY104,LEU392, ASN394, LYS562 **Pi-alkyl:** PHE40, TRP69, LEU73, ALA99, PHE390, LEU391
7	Withanone Binding Energy:-8.5		**Convention hydrogen bonding:** ALA348 **Van der vaals:** SER44, SER47, LEU351, HIS378, HIS401, FLU402 **Pi-alkyl:** TRP349 **Pi-Sigma:** PHE40
8	Beta-sitosterol Binding Energy:-8.3		**Convention hydrogen bonding:** LEU100 **Van der vaals:** PHE32, TRP69, SER77, ALA99, GLN102, ASP350, TYR385, LEU391, ASN394 **Pi-alkyl:** PHE40, PHE73, PHE390, ARG393
9	Campesterol Binding Energy:-8.3		**Convention hydrogen bonding:** LEU100 **Van der vaals:** PHE32, TRP69, SER77, ALA99, ASP350, GLY352, ASN394 **Pi-alkyl:** PHE40, LEU73, TYR385, PHE390, LEU391, ARG393
10	Orientin Binding Energy:-8.0		**Convention hydrogen bonding:** PHE390, ARG393 **Van der vaals:** PHE40, SER43, SER44, SER47, ASP350, ASP382, TYR385, LEU391, ASN394, HIS401

Table 5: 2D interaction of top 12 phytochemicals with human furin (PDB ID: 5MIM)

S. No.	Name of the molecules	2D interaction between molecules and protein	Kinds of the binding
1	Native ligand, 1N Binding energy= -9.3		**Convention hydrogen bonding:** LEU227, GLY255, PRO256, ASP258, THR308, THR365 **Van der vaals:** ARG185, ASN192, GLY229, SER253, TRP254, GLU257, GLY265, ASN295, HIS364,GLY366, SER368, **Pi-alkyl:** VAL231 **Attractive charge:** ASP154, ASP191, ASP228, ASP258, ASP264
2	Withanolide Binding energy=-9.5		**Convention hydrogen bonding:** PRO266, ASN29, ALA532 **Van der vaals:** VAL263, ASP264, GLY265, ALA267, GLU271, GLY307, ASN310, SER311, ARG498, ASP526, GLY527, PHE528 **Pi-alkyl:** TRP531
3	Somniferine A Binding energy=-8.7		**Convention hydrogen bonding:** GLY255 **Van der vaals:**LEU227, SER253, TRP254, ASP258, ASP259, GLY294, ASN295, GLY296,ARG298, GLU299, TRP328, SER368 **Carbon hydrogen bond:** ALA292, ASP306
4	Apigenin Binding energy=-8.5		**Convention hydrogen bonding:** GLU271, ASN310, ALA532 **Van der vaals:** GLY265, PRO266, GLY307, SER311, ILE312, TYR313, GLN488, MET534 **Pi-alkyl:** ALA532 **Pi-Pi:** TRP531

5	Withanolide B Binding energy=-8.3		**Convention hydrogen bonding:** SER330 **Van der vaals:** GLY297, HIS300, ASP301, ASN325, TRP328, GLU331, ALA332, ASN407, ALA408, ASN409, SER423 **Pi-alkyl:** VAL326
6	Ursolic acid Binding energy=-8.3		**Convention hydrogen bonding:** ASP154, ASN192, HIS194, GLY255 **Van der vaals:** GLU236, PRO256, GLU257 **Pi-alkyl:** LEU227, VAL231, TRP254
7	Hesperetin Binding energy=-8.2		**Convention hydrogen bonding:** PRO266, GLU271, ASN310, ALA532 **Van der vaals:** GLY265, ALA267, PHE274, GLY307, SER311, ILE312, TYR313, GLN488, MET534 **Pi-alkyl:** TRP531
8	Campesterol Binding energy=-8.1		**Van der vaals:** ASP264, GLY265, GLU271, GLY307, ASN310, SER311, GLN488, ARG498, GLY527, PHE528, ASN529, ASP530 **Pi-alkyl:**VAL263, PRO266, TRP531, ALA532
9	Crategolic acid Binding energy=-8.1		**Convention hydrogen bonding:** ASP154 **Van der vaals:** ASP153, ASP191, ASP192, GLU236, GLY255, PRO256, GLU257, ASP258 **Pi-alkyl:** HIS194, LEU227, VAL231, TRP254

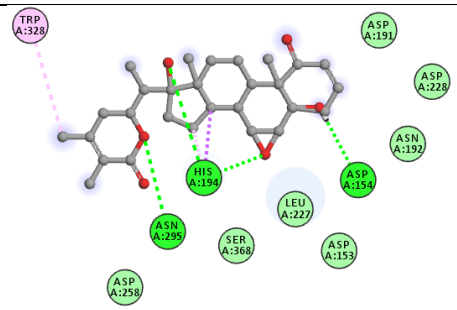
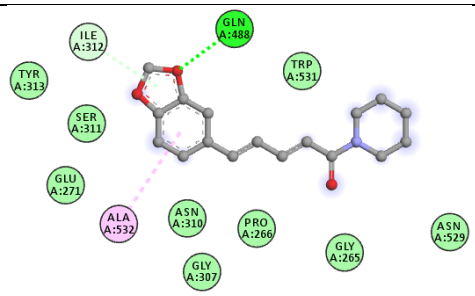
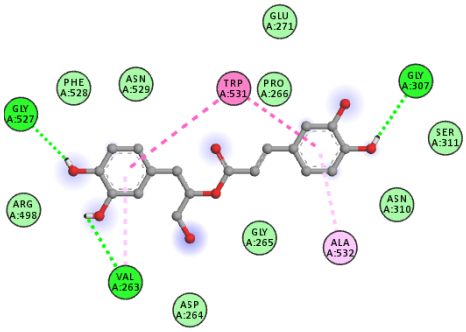
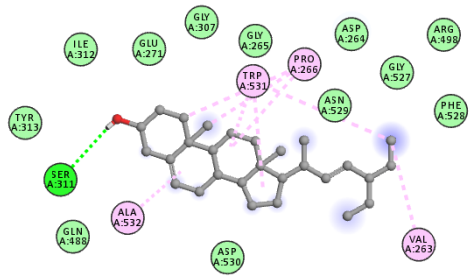
10	Withanone Binding energy=-8.1		**Convention hydrogen bonding:** ASP154, HIS194, ASN295 **Van der vaals:** ASP153, ASP191, ASN192, LEI227, ASP228, ASP258, SER368 **Pi-alkyl:**TRP328 **Pi-Sigma:** HIS194
11	Chavicine Binding energy=-8.0		**Convention hydrogen bonding:** GLN488 **Van der vaals:** GLY265, PRO266, GLU271, GLY307, ASN310, SER311, ILE312, TYR313, ASN529,KTRP531 **Pi-alkyl:** ALA532
12	Rosmarinic acid Binding energy=-8.0		**Convention hydrogen bonding:** VAL263, GLY307, GLY527 **Van der vaals:** ASP264, GLY265, PRO266, ASN310, SER311, ARG498,PHE528, ASN529 **Pi-alkyl:** ALA532 **Pi-Pi:** TRP531
13	Stigmasterol Binding energy=-8.0		**Convention hydrogen bonding:** SER311 **Van der vaals:** ASP264, GLY265, GLU271, GLY307, ILE312, TYR313, GLN488, ARG498, GLY527, PHE528, ASN529, ASP530 **Pi-alkyl:** VAL263, PRO266, TRP531, ALA532

Table 6							
Phytochemicals	**Target molecules; binding energy (kcal/mole) (Only those phytochemcals are included in the table which have binding energy <- 7.5)**						
	6vww (SARS-CoV-2 NSP15 Endoribonuclease)	**6w02 (SARS-CoV-2 ADP ribose phosphatase of NSP3)**	**6vyo (SARS-CoV-2 nucleocapsid RBD)**	**6w4b (SARS-CoV-2 Nsp9 RBP)**	**4mds (SARS-CoV 3CLpro)**	**2ajf (SARS-CoV spike)**	**2cjr-SARS-CoV Nucleocapsid**
1-dehydrogingerdione	-7.5	-7.5					
6-gingerdione		-7.5					
6-gingerol		-7.5					
Apigenin	-9	-8.7					
Berberine	-8.2	-9.7					
Beta-sitosterol	-8.6	-7.6		-8		-8	
Bisdemethoxycurcumin		-8.8				-7.6	
Brachyamide B	-7.7	-8.1					
Calebin A		-8.1			-8	-7.6	
Campesterol	-8.5	-7.9		-8	-7.7	-7.6	
Chavicine	-7.6	-8.8					
Cirsilineol	-7.6	-8.3					
Cirsimaritin	-7.6	-8.8					
Cordifolioside A	-7.7						
Cordioside	-8.1				-8.1		
Crategolic acid	-9.1	-7.5	-7.7		-8.1		
Curcumin	-8.6	-8.2					
Cyclocurcumin	-8.3	-9.2			-7.7	-7.6	
Demethoxycurcumin		-8.8			-7.6		
Eriodictyol	-9	-8.4					
Eugenitin		-7.6					
Flavonol glucoside	-8.5	-8.6		-7.5			
Guineensine		-7.8					
Hesperetin	-9	-8.5					
Isochavicine		-7.5					

Isopiperine	-8.1	-7.8					
Isothymonin	-7.6						
Isothymusin	-7.6	-8					-7.5
Jatrorrhizine	-7.9				-7.5		
Kaempferol	-8	-8.6					
Magnoflorine	-7.9						-8.2
Oleanolic acid	-9	-7.7	-7.6		-8.2	-7.9	-8.4
Orientin	-9.4	-8.2			-7.8		-7.7
Pentadienoylpiperidine	-8.7	-8.6			-7.8	-7.9	
Piperamine		-7.7					
Piperettine	-7.9	-9.2			-7.5	-7.9	
Pipericide		-7.6					
Piperine	-8.7					-7.6	
Piperolein B		-8.7					
Quercetin	-8.1	-8.3	-7.7			-7.5	-7.6
Retrofractamide A	-7.9	-7.8				-7.6	
Rhamnetin	-7.9	-8.4					-7.6
Rosmarinic acid	-7.5	-9.3			-7.7		
Somniferine A	-8.5	-8.3	-8.2	-7.5	-9.3	-8.5	-8.6
Stigmasterol	-8.9	-7.9	-7.7	-7.9	-7.9	-8.6	
Tinocordifolin	-7.5	-7.6					
Tinocordioside			-7.7		-7.5		
Tinosporaside	-7.6		-7.7		-8.1		
Tinosporide	-8.1	-8.2			-7.7	-7.8	
Tricholein		-8.1					
Trichostachine	-8.4	-8.6			-7.5	-7.6	
Umbelliferone		-7.5					
Ursolic acid	-9.1	-7.6	-7.8		-8	-8.2	-9
Vicenin	-7.8	-7.6					
Withaferin A	-9.1	-9.2	-8.3	-8.2	-7.7		-7.7
Withanolide	-9.3	-9.6	-8.7	-8.1	-8.3	-8.7	-7.9
Withanolide B	-9.2	-9.2	-8	-9	-8.9	-8.6	-8.2
Withanone	-8.9	-8.3	-8.2	-9.1	-8.5	-7.8	-7.8

Table 7

Compounds	Target molecules; binding energy (kcal/mole) (Only those phytochemcals are included in the table which have binding energy <-8.0)			
	COX2 (PDB ID: 5f1a)	**PLA2 (PDB ID:4uy1)**	**NIK(PDB ID:4dn5)**	**IRAK-4 (PDB ID:2nru)**
Native ligand	-6	-7.7	-6.9	-9.4
Phytochemicals				
Apigenin	-9.2		-9.5	-8.3
Berberine	-9	-8.4		-9
Beta-sitosterol	-8.7			-9.7
Bisdemethoxycurcumin	-9.4		-8.5	-8.6
Brachyamide B			-8.4	-8.7
Calebin-A	-9.1			-8.5
Campesterol	-8.7	-8.1	-9.5	-9.9
Chavicine	-8.2	-8.4		-8.3
Cirsilineol	-8.8		-8.8	-8.4
Cirsimaritin	-8.9		-9.1	-8.4
Cordifolioside A	-8.2			
Cordioside	-9.6			
Crategolic acid	-8.9	-8.3	-8.1	
Curcumin	-8.2		-8	-8.4
Cyclocurcumin	-9.3	-8.4	-8.8	-9.4
Demethoxycurcumin	-8.9	-8		-8.9
Eriodictyol	-9.7	-8.1	-9.7	-8.7
Eugenitin	-8			
Flavonol glucoside	-8.9			-8.7
Guineensine				-8.7
Hesperetin	-8.9		-9.4	-8.4
Isochavicine	-9.3	-8.7		-8.9
Isopiperine	-8.6	-8.1	-8.7	-8.9
Isothymonin	-9		-8.8	-8.3
Isothymusin	-8.7		-8.9	-8.4
Jatrorrhizine	-9.1			-9
Kaempferol	-8.9		-8.6	-8.1
Magnoflorine	-8.4			-9.3
Oleanolic acid	-8.9	-8	-8.2	-9.2
Orientin	-9.6	-8.9	-8.6	-9.5
Pentadienoylpiperidine	-8.7			-8.3
Piperamine	-8.1			-8.3
Piperettine		-8.5		-9.1
Piperine	-8.7	-8.5		-8.1
Piperolein b	-8.6			
Quercetin	-9.6	-8	-9.1	-8.4
Retrofractamide A				-8
Rhamnetin	-9.1	-8	-9.3	-8.9

Rosmarinic acid	-9.4	-9.1		-8.7
Somniferine A	-9.2	-8.5	-8.6	-8.4
Stigmasterol	-9.6	-8	-8.2	-9.8
Tinocordifolioside	-8.1			-9.6
Tinocordioside	-8.4	-8.1		
Tinosporaside	-8			
Tinosporide			-8	
Trichostachine	-8.5	-8.1		
Ursolic acid	-8.9			
Vicenin	-8.8		-8.5	-10.1
Withaferin A	-8.8	-9.1	-8.7	-9.2
Withanolide	-9.4	-9.7	-8.6	-8.2
Withanolide B	-9.3	-9.4	-8.4	-11.5
Withanone	-10		-8.1	-8.7