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Evaluation of traditional ayurvedic Kadha for prevention and management of the novel Coronavirus (SARS-CoV-2) using in silico 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 (COVID-19) patients has demonstrated the high transmission rate among the human population. 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 the prevention and treatment of COVID-19 by re-evaluating the knowledge of traditional medicines and repurposing of drugs. Here, we used molecular docking and molecular dynamics simulation approach to explore the beneficial roles of phytochemicals and active pharmacological agents present in the Indian herbs 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. 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. The phytochemicals present in these herbs possess significant anti-inflammatory property. Apart from this, based on their pharmaceutical characteristics, we have also performed in-silico drug-likeness and predicted pharmacokinetics of the selected phytochemicals found in the Kadha. 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 COVID-19.

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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 COVID-19. 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 (Salata et al., 2019; Ye et al., 2020).

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 the 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 COVID-19 (Wu et al., 2020). Among these, repurposing approach will shorten the time

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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 COVID-19 both as prophylaxis and therapeutic purpose.

Ayurvedic medicines and their extracts are used for the treatment of viral diseases for a very long time (Alleva et al., 2010; Arora et al., 2011). Ayurvedic medicines are used in Indian subcontinent since the Vedic period dating back to more than 5000 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) and (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 recommended the use of Kadha for boosting the immunity and reducing inflammation during COVID-19 crisis (The Ministry of AYUSH 2020). To manage COVID-19 crisis, several Indian Ayurvedic herbs, spices and their active phytochemicals have been explored for their possible prophylactic and therapeutic use against COVID-19 (Chowdhury, 2020; Emirik, 2020; Kumar et al., 2020; Sen et al., 2020; Shree et al., 2020; Swargiary et al., 2020; Tripathi et al., 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., 2018; Takeuchi & 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 & 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 & Korteweg, 2007; Wang et al., 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 & Korteweg, 2007; Wang et al., 2013). Clinical investigations 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 the functional region of viral proteins including spike, proteases, nucleoproteins and polymerase as well as host surface receptors. These phytochemicals also showed a 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 the preparation of Kadha or similar drinks were collected from the 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., 2010), 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 and Tinocordifolin) (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 & 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 & Tyagi, 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) (Cortes-Rojas et al., 2014), Elettaria cardamomum (Protocatechualdehyde, Protocatechuic acid, Alpha-terpinyl acetate, 1,8-cineole, Linalool, Linalyl acetate, Limonene, 4-terpineol and Geraniol) (Noumi et al., 2018), Citrus limon (Eriodictyol, Quercetin, Hesperetin, Phloroglucinol, Umbelliferone, vitamin C (Vandercook & Stephenson, 1966; Rangel et al., 2011) and Withania somnifera (Withaferin A, Somniferine, Choline, Anaferine, Withanolide A, Withanolide B, Withanone and Withanolide) (Sangwan et al., 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 the availability of ingredients. The most common ingredients are Tulsi leaves (10–15 leaves or ¼ teaspoon powder), Ginger (2–5 g rhizome or ¼ teaspoon powder), Clove (4–5 pieces), Black pepper (4–5 pieces), Cardamom (4–5 pieces), Ashwagandha (2–5 g raw or ¼ teaspoon powder) and Giloy (2–5 g raw or ¼ teaspoon powder). To make the Kadha, these herbs are boiled in 200 ml water for 5–10 min, 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

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 the following PDB ID's 6LU7 (SARS-CoV-2 main protease, Mpro), 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). To study the interaction of different phytochemicals with different pro-inflammatory mediators like COX2, PLA2, NIK and IRAK-4, we have used the following PDB ID's, 5f1a, 4uy1, 4dn5 and 2nru, respectively. All the protein structures were retrieved from the 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 were used for preparation of the input files and performing molecular docking using Vina wizard (Dallakyan & Olson, 2015; Oleg & Olson, 2010). 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 Mpro 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 Mpro (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.

Molecular dynamics simulation

The structures of protein-ligand complexes comprising of SARS-CoV-2 Mpro or human ACE2 receptor protein bound with selected phytochemicals were obtained by molecular docking. The output files containing protein-ligand complexes were used for molecular dynamic (MD) simulation using GROMACS (Abraham et al., 2015). The topology files for protein and ligand were prepared using pdb2gmx module of GROMACS and Prodrg server (http://prodrg1.dyndns.org/), respectively (van Aalten et al., 1996). We used Gromos53a6.ff Force-field during the MD simulation as described previously (Maurya, 2020). In brief, proteins were solvated using simple point charge (SPC) water model and counter ions were added to neutralize the system. The system was energy minimized and water and ions were allowed to equilibrate around the protein in a two-step equilibration process. The first part of equilibration was carried out for 100 picoseconds at a constant number of particles, volume and temperature (NVT). The second part of equilibration was carried out for 100 picoseconds with a constant number of particles, pressure and temperature (NPT). Following equilibration, the production simulation duration was performed for 25 nanoseconds. All the simulation runs were carried out in triplicates and mean values were graphed using Excel. After completion of MD simulation, results for flexibility of protein-ligand complexes were analyzed using root

mean square deviation (RMSD) based on whole protein and root mean square fluctuation (RMSF) based on the C-alpha using GROMACS.

MM-PBSA binding free energy calculation

After MD simulation, in order to have an enhanced ranking of the ligands and determination of their predictive binding energies, Mechanic/Poisson-Boltzmann Surface Area (MM-PBSA) calculations were implemented in MD output files. The binding free energy provides an overview of the bio-molecular interactions between protein and ligand which constitutes of potential energy, polar and non-polar solvation energies. More negative binding free energy values indicate a stronger binding. Here in this study, MM-PBSA binding free energies were calculated using a GROMACS script 'g_mmpbsa' (Kumari et al., 2014). The binding energy was calculated using the following equation:

$$\Delta\ G_{binding} = G_{complex} - (G_{receptor} - G_{ligand})$$

where, Δ Gbinding represents the total binding energy of the complex, binding energy of free receptor is $G_{receptor}$ and that of unbounded ligand is represented by G_{ligand}.

In-silico drug-likeness, pharmaceutical characterization and pharmacokinetics

We sought to predict in-silico drug-likeness and important pharmaceutical properties including pharmacokinetics of the selected phytochemicals which exhibited high affinity for different SARS-CoV-2 viral proteins and human ACE2 and Furin proteins. For this purpose pkCSM online prediction platform was used (Pires et al., 2015). This online server calculates pharmaceutically applicable properties such as molecular weight, octanol-water partition coefficient (LogP), number of H-bond donor, number of H-bond acceptor and number of rotatable bonds. This platform was also used to estimate additional parameters like ligand Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET), water-solubility, human intestinal absorption, total clearance, AMES test, human maximum tolerated dose and hepatotoxicity.

Results

Our molecular docking study revealed that different phytochemicals found in the traditional Ayurvedic Kadha may have a 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/mol) with SARS-CoV-2 Mpro, spike protein, human ACE2 and furin proteins.

Supplementary material Table S1 shows the predicted binding of top 12 phytochemicals and native ligand N3, against SARS-CoV-2 Mpro 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/mol, respectively. All these molecules share the same binding site with the well-known inhibitor, N3, and their binding energy is also comparable (Figure 1).

SARS-CoV-2 spike protein interacts with the host ACE2 receptor present on the surface of the host cells for their entry. Supplementary material Table S2 shows the binding of the 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/mol, respectively. These phytochemicals were found to share the same binding pocket in the target proteins (Figure 2a).

Supplementary material Table S3 shows the binding of the 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/mol, respectively. These phytochemicals share the same binding pocket in human ACE2 (Figure 2b).

Furin is another protease found in the host cells which acts on the viral spike protein and facilitates its interaction with the human ACE2. Supplementary material Table S4 shows the predicted binding of the 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/mol, respectively. Many of these molecules share the 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 the binding site of native ligand (Figure 3).

The molecular docking performed against other proteins from SARS-CoV-2 and SARS-CoV-1 proteins are shown in Supplementary material Table S5. Many of the phytochemicals present in the Kadha have a significant binding affinity with SARS-CoV-2 and SARS-CoV-1 proteins. Some of the phytochemicals which have a 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/mol, respectively. A list of the phytochemicals

Table 1. The predicted binding of different phytochemicals against SARS-CoV-2 main protease, spike protein, human ACE2 and furin proteins.

	Target molecules; binding energy (kcal/mol) (Only those phytochemicals are included in the table which have binding energy ≤ −7.5)			
Compounds	COVID-19 Mpro (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

Only those phytochemicals name are included in the table which have binding energy ≤ 7.5 kcal/mol.

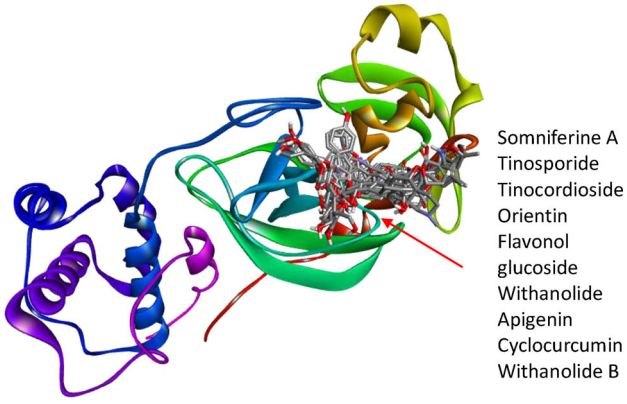


Figure 1. SARS-CoV-2 Mpro (PDB ID: 6LU7) showing top 12 phytochemicals superimposed on it active site.

which were found to exhibit high affinity with SARS-CoV-2 ADP ribose phosphatase, NSP3 (PDB ID: 6w02) is 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/mol, 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/mol, respectively.

More than ten phytochemicals were predicted to interact with the 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

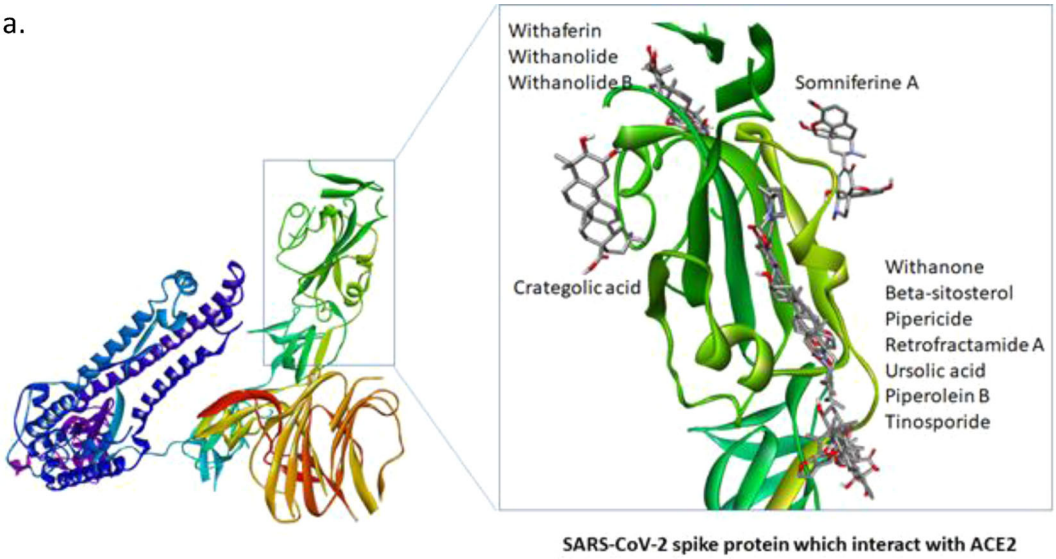
Table 2. Binding energy of different phytochemicals with molecules involved in the inflammatory processes.

	Target molecules; binding energy (kcal/mol) (Only those phytochemicals are included in the table which have BE <- 8.0)			
Compounds	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
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
Flavonol glucoside	−8.9			−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
Quercetin	−9.6	−8	−9.1	−8.4
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
Withaferine 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

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/mol, respectively.

An array of the phytochemicals (having binding energy ≤ 7.5 kcal/mol) 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/mol, respectively. Similarly, the phytochemicals (having energy ≤7.5 kcal/mol) 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 −7.5 kcal/mol, 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/mol, respectively.

Table 2 shows the predicted binding energy of different phytochemicals with molecules involved in the inflammatory



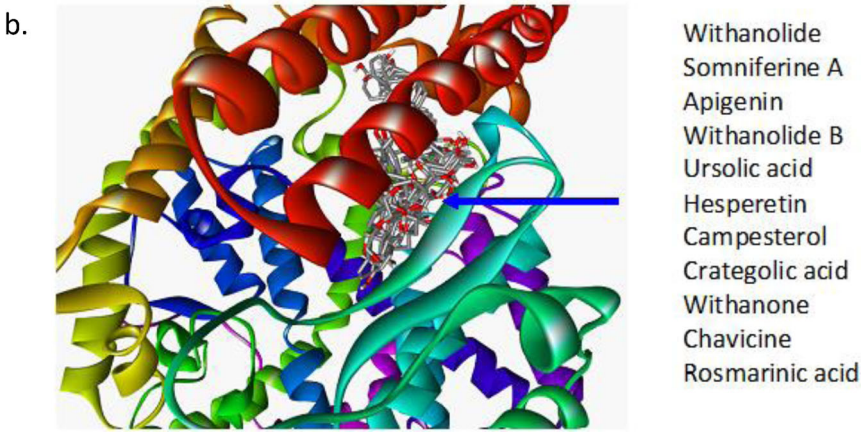


Figure 2. Selected phytochemicals superimposed on SARS-CoV-2 spike and human ACE2. (a) SARS-CoV-2 spike, (b) Human ACE2.

Table 3. MM-PBSA binding energies with Mpro.

Molecules ->	Orientin		Somniferine		Tinocordioside		Tinosporide		Withanolide	
Energy	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
van der Waal energy	−130.34	5.14	−128.84	40.59	−112.73	17.11	−136.47	6.77	−112.22	17.32
Electrostatic energy	−48.80	1.16	−527.13	60.52	−52.55	14.66	−13.11	2.11	−31.34	14.51
Polar solvation energy	115.52	2.02	361.79	39.11	117.61	16.1	65.93	4.12	91.06	20.46
SASA energy	−13.75	0.16	−13.71	3.16	−13.26	2.17	−12.99	0.41	−11.6	1.96
Binding energy	−77.38	4.4	−191.8	181.26	−60.93	17.85	−96.64	7.07	−22.81	9.31

Table 4. MM-PBSA binding energies with ACE2.

Ligands name ->	Somniferine		Withaferine A		Withanolide		Withanolide B		Vicenin	
Energies	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
van der Waal energy	−163.67	33.25	−130.88	37.36	−179.83	8.21	−146.57	5.045	−90.40	20.10
Electrostatic energy	−1151.9	141.61	−26.58	10.03	−44.23	4.14	−17.09	3.21	444.5	53.28
Polar solvation energy	282.98	59.92	98.78	14.33	130.88	4.11	64.84	5.20	125.1	26.64
SASA energy	−15.53	3.31	−14.01	3.98	−18.85	0.47	−14.43	0.43	−10.89	2.62
Binding energy	−1048.11	122.09	−72.69	37.46	−112.03	9.59	−113.25	2.42	468.4	55.4

processes such as COX2, PLA2, NIK and IRAK-4. From Table 2, 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 a 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

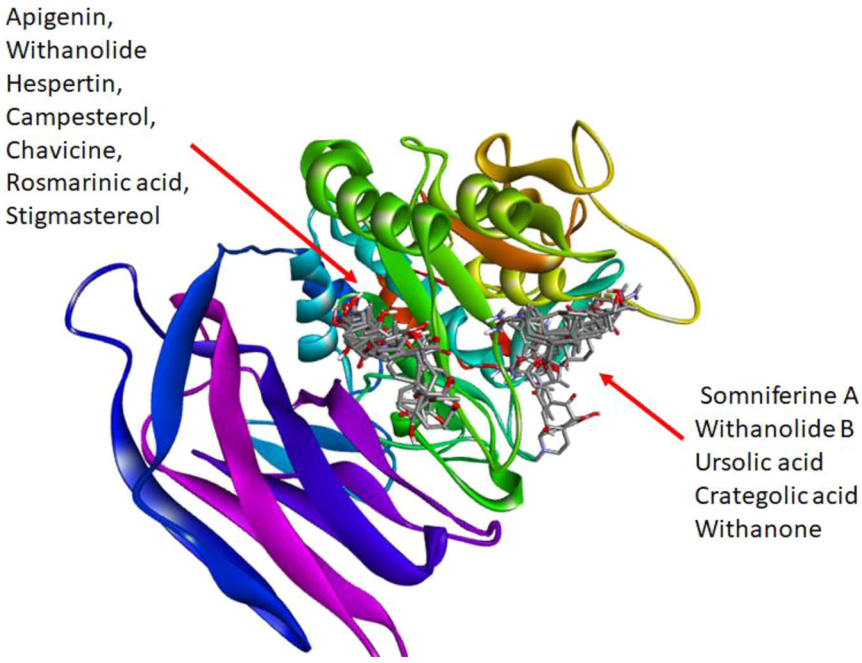


Figure 3. Selected phytochemicals superimposed on human furin (PDB ID: 5MIM).

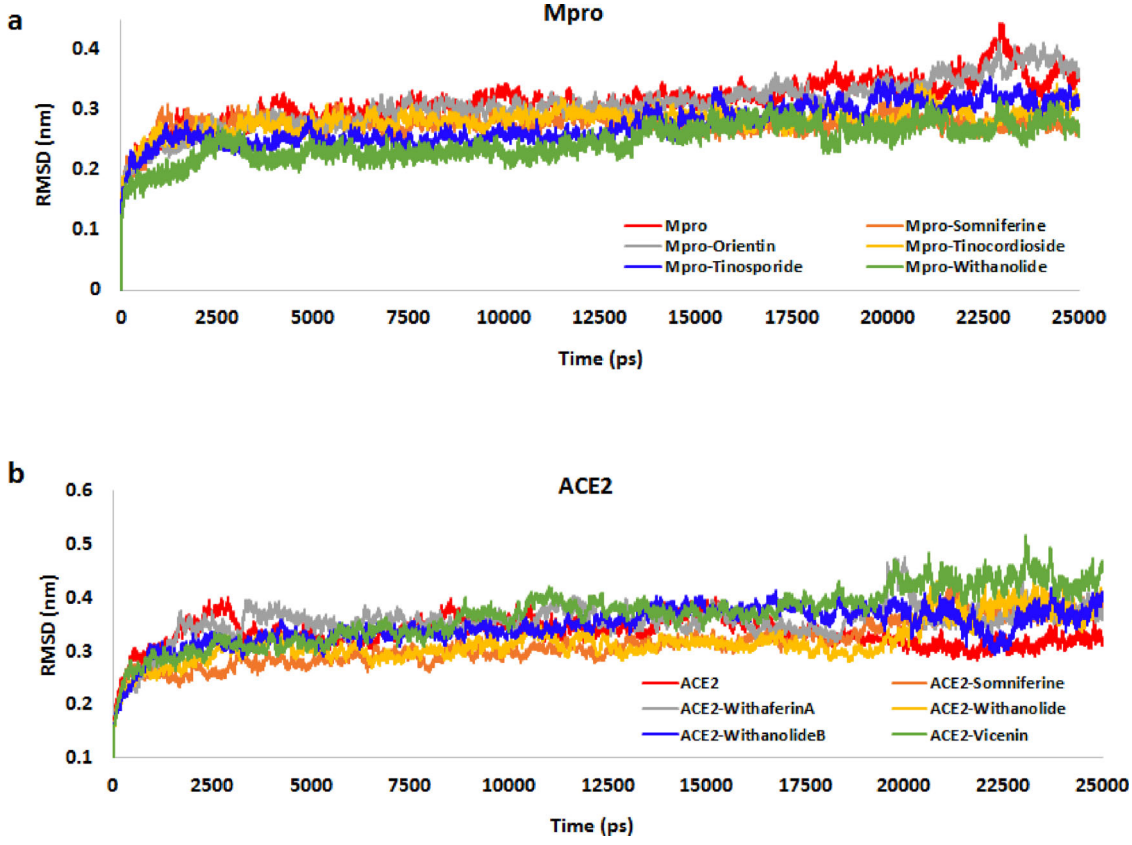


Figure 4. RMSD plot of SARS-CoV-2 Mpro and human ACE2 protein. (a) Mpro alone or complexed with Orientin, Tinosporide, Tinocordioside, Somniferine A and Withanolide, (b) ACE2 alone or complexed with Withaferine A, Withanolide, Withanolide B, Somniferine A and Vicenin.

bind to the active site of the protein and at the same time, few of them may have a 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.

Figure 4 shows the MD simulation results carried out with selected phytochemicals and proteins. MD simulations were

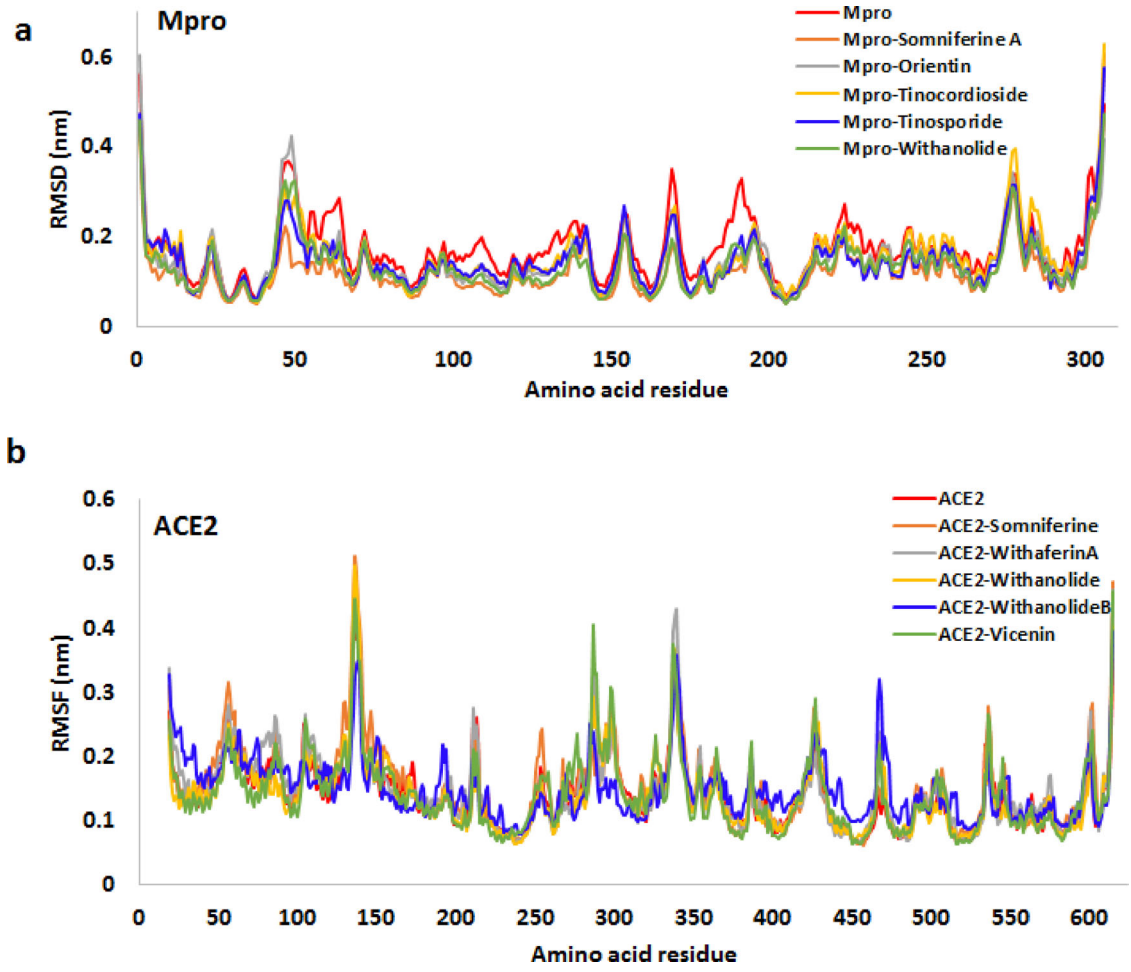


Figure 5. RMSF plot of SARS-CoV-2 Mpro and human ACE2 protein. (a) Mpro alone or complexed with Orientin, Tinosporide, Tinocordioside, Somniferine A and Withanolide, (b) ACE2 alone or complexed with Withaferine A, Withanolide, Withanolide B, Somniferine A and Vicenin.

carried out for SARS-CoV-2 Mpro and human ACE2 receptor with selected phytochemicals in the solvated states for 25 ns. We have selected Mpro viral protein because it is a crucial druggable target in SARS-CoV-2. Phytochemicals Somniferine A, Tinosporide, Tinocordioside, Orientin and Withanolide complexed with Mpro were employed for the MD simulation. The second target protein selected in our study was the human ACE2 receptor protein because it is responsible for the interaction with the viral spike protein. Phytochemicals Tinosporide, Withaferine A, Vicenin, Withanolide B, Withanone complexed with ACE2 were employed for the MD simulation. The RMSD plot analysis indicates that for both target proteins, the RMSD values showed an initial steady increase and then reached equilibrium during the rest simulation period (Figure 4a and b). However, we also noticed a few variations that were of the order of about 0.15 nm which indicated that the complex did not undergo large conformational changes. To explore the local protein flexibility, the time average of RMSF values of Mpro and ACE2 protein in the presence of different phytochemicals over the simulation period was calculated using C-alpha of proteins. RMSF with respect to amino acid for both Mpro and ACE2 were plotted and we did not find any major fluctuation except for a few places where moderate fluctuation was observed (Figure 5a and b). The output of the MD simulation was further used for MM-PBSA calculation utilizing g_mmpbsa package and python script MmPbSaStat.py and MmPbSaDecomp.py which calculates average free binding energy and residual energy contribution of the complex, respectively. Tables 3 and 4 represent the calculated MM-PBSA binding energy for different phytochemicals with Mpro and ACE2, respectively. Except for the polar solvation energy, all other forms of energy contributed favourably to the interaction between different phytochemicals with Mpro and ACE2. Binding energy obtained from MM-PBSA calculation corroborated positively with the findings of molecular docking study.

We also examined the contribution of individual residues from Mpro and ACE2 proteins towards overall binding free energy involved in the interaction with selected phytochemicals. It was found that the residues THR25, LEU27, MET49, CYS145, GLU166, LEU167 and ASP176 of Mpro are major contributors towards binding with Tinosporide. The CYS145, HIS164, MET165, LEU167, PRO168 and GLN189 residues contributed towards the binding of Mpro with Orientin. The residues THR25, LEU27, THR45, MET49, LEU141 and CYS145 contributed significantly towards the binding of Mpro with Tinocordioside. Withanolide binding was mainly through the residues PRO52, TYR54, CYS85, PHE181, PRO184, VAL186,

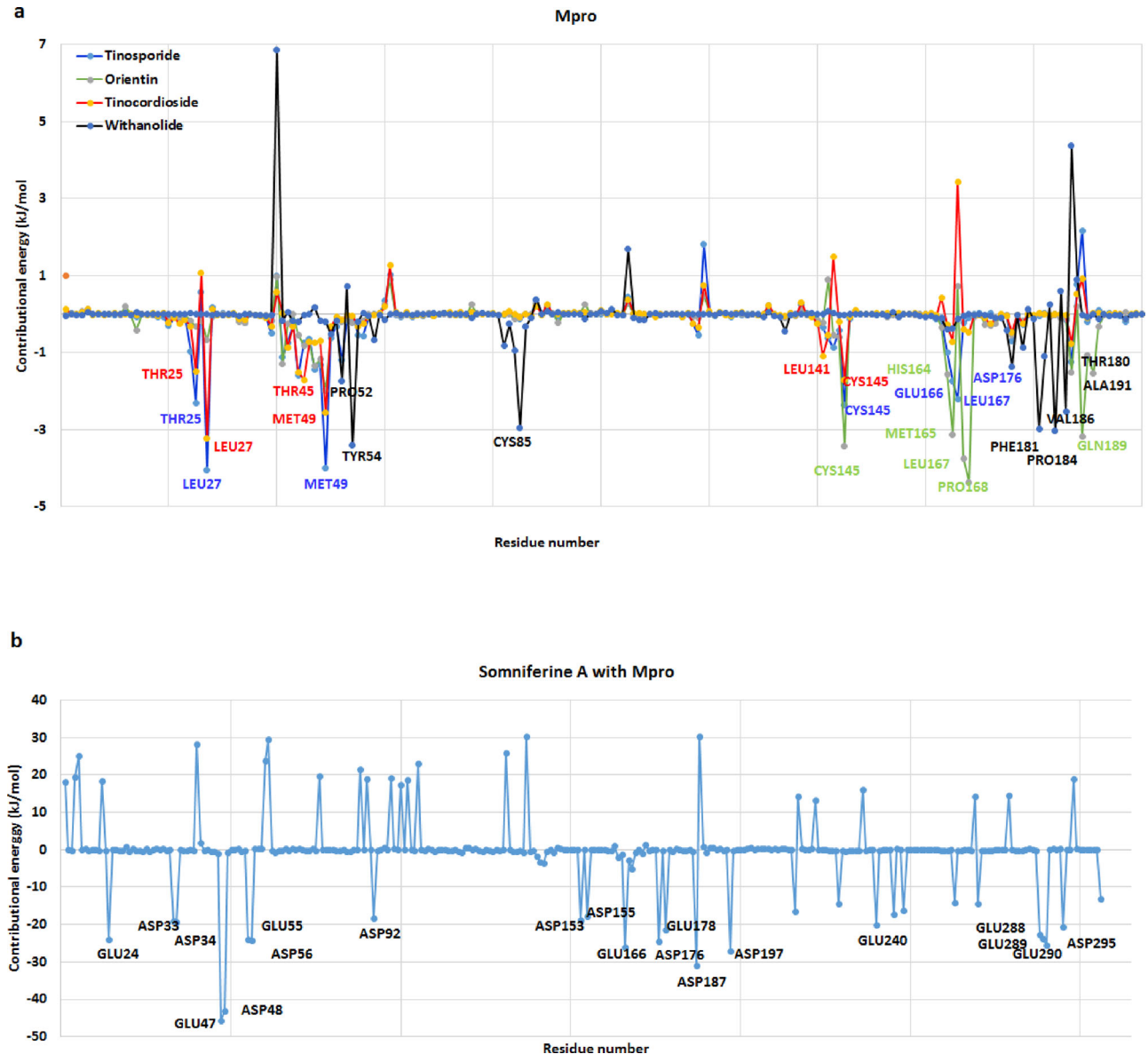


Figure 6. Graphical representation of per residue energy contribution plot for against SARS-CoV-2 Mpro. (a) Tinosporide (blue), Orientin (green), Tinocordioside (red) and Withanolide (black), (b) Somniferine A.

THR180 and AL191 of Mpro (Figure 6a). Among the five phytochemicals used in our study, Somniferine showed the highest number of interacting residues with the Mpro. Some of the amino acids residues which significantly contribute towards binding energy between Mpro and Somniferine include GLU24, ASP33, ASP34, GLU47, ASP48, GLU55, ASP56, ASP92, ASP153, ASP155, GLU166, ASP175, GLU178, ASP187, ASP197, GLU240, GLU288, GLU289, GLU290 and ASP295 (Figure 6b).

Similarly, the energy contribution of each amino acid residue of ACE2 with various phytochemicals was examined (Figure 7a and b). For Withaferine A, TRP69, LEU73, PHE390 and LEU391 were identified as major energy contributing to amino acid residues. For Withanolide, the residues which were involved in interaction were GLU22, GLU37, PHE40, LEU85, LEU91, LEU95, LEU100, HIS195, TYR196, ASP201 and VAL209. The phytochemical Withanolide B significantly interacted with amino acid residues PHE40, TRP69, LEU73, LEU100, ASP350, PHE390 and LEU191 of ACE2. We also found that Somniferine A interacted with the huge number of amino acids residue in ACE2 similar to Mpro (Figure 7b). Therefore, we propose Somniferine A possible utility for the management of COVID-19.

The phytochemicals which were predicted to bind with different SARS-CoV-2 proteins and human ACE2 & Furin proteins were analyzed for their in-silico drug-likeness and pharmacokinetics using pkCSM server (Pires et al., 2015) (Supplementary material Tables S6 and S7). Some of these phytochemicals did not follow the Lipinski's rule of five for all the parameters. The ADMET properties derived from pkCSM server indicated reasonable intestinal absorption and bioavailability of the phytochemicals such as Apigenin, Berberine, Beta-sitosterol, Bisdemethoxycurcumin, Brachyamide B, Campesterol, Chavicine, Crategolic acid, Curcumin, Cyclocurcumin, Demethoxycurcumin, Eriodictyol, Isochavicine, Isopiperine, Isothymonin, Jatrorrhizine,

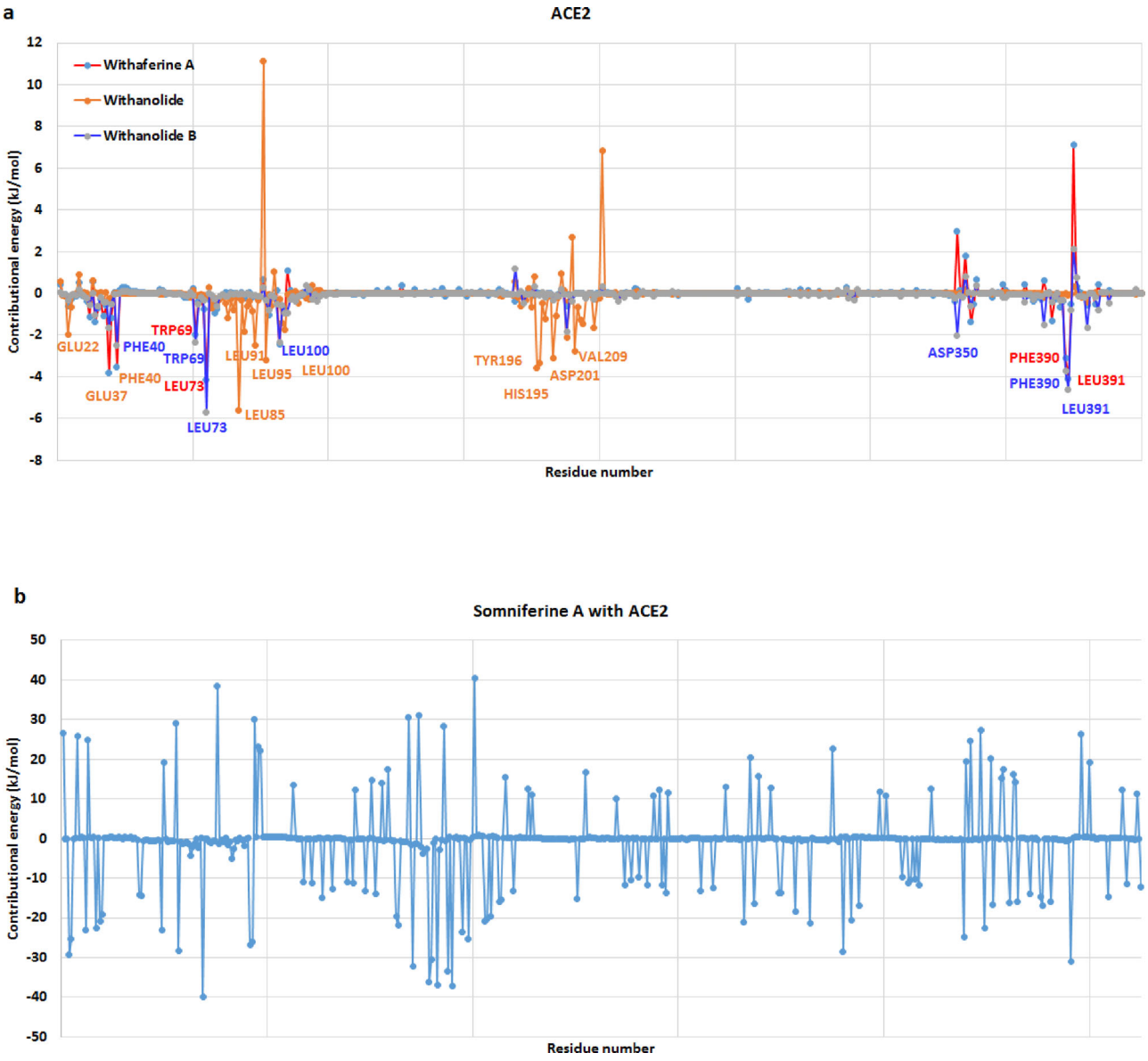


Figure 7. Graphical representation of per residue energy contribution plot for against human ACE2. (a) Withaferine A (red), Withanolide (orange) and Withanolide B (blue), (b) Somniferine A.

Kaempferol, Oleanolic acid, Piperettine, Pipericide, Piperolein B, Quercetin, Retrofractamide A, Rhamnetin, Somniferine A, Stigmasterol, Tinocordioside, Tinosporide, Trichostachine, Ursolic acid, Withaferin A, Withanolide, Withanolide B and Withanone are significantly very high (>70%). Among these phytochemicals, only Berberine was shown to be negative for AMES test (Supplementary material Table S7).

Discussion

The use of herbs and phytochemicals has a long history in the management of various respiratory diseases (Alamgeer et al., 2018; Pinn, 2001; Santana et al., 2016). Currently, the demand of complementary medicine, including herbal medicine has become more popular in healthcare for both general maintenances of health and treatment of minor illnesses (Barnes, 2004). In European countries, several species of herbs have been used against the flu and common cold (Weiss & Fintelmann, 2000). Similarly, in Russia and Estonia various herbs and medicinal plants have also been used for centuries for the management of common cold and flu (Raal et al., 2013). In India, the use of spices and herbs for the treatment of various diseases including cough, cold is common practice with a recorded history of over 2000 years (Sachan et al., 2018; Vasanthi & Parameswari, 2010). A number of Ayurvedic herbs have been used for management of SARS-CoV-2 infection (Shree et al., 2020; Tripathi et al., 2020). Some of the key Indian herbs such as Ashwagandha, Tulsi, Giloy and Turmeric contain several active phytochemicals which selectively bind with various SARS-CoV-2 vital targets (Chowdhury, 2020; Emirik, 2020; Kumar et al., 2020; Sen et al., 2020; Shree et al., 2020; Tripathi et al., 2020). The phytochemicals from several Indian spices have also shown possible beneficial effect against SARS-CoV-2 (Sen et al., 2020).

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 the release of 16 non-structural proteins (NSPs) (Jo et al., 2020). Thus SARS-CoV-2 Mpro 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 a significant binding affinity with the Mpro. 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 (Figure 1, Supplementary material Table S1). The binding of these phytochemicals with Mpro 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 drugs (Li, 2016). Our study predicts that an array of the phytochemicals have significant binding affinity with the SARS-CoV-2 spike proteins (Supplementary material Table S2, Figure 2a) as well as with host ACE2 protein (Supplementary material Table S3, Figure 2b) and furin protein (which facilitate spike and ACE2 interaction) (Supplementary material Table S4, 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., 2017).

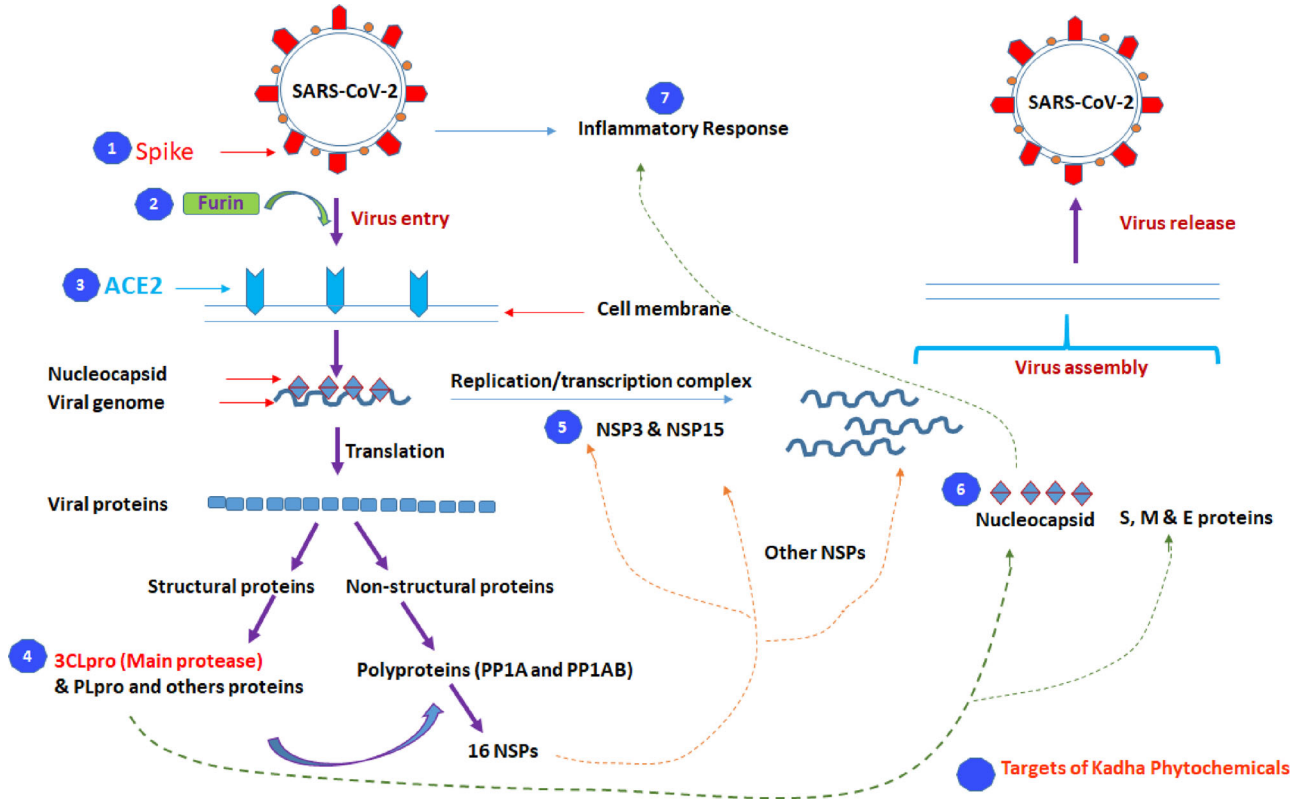
SARS-CoV nucleocapsid protein (SARS-CoV NP) is another vital structural protein which shows intrinsic multimerization and interacts with M protein, suggesting that NP is both critical to the formation of the viral nucleocapsid core and is involved in virion assembly (He, Dobie et al., 2004; He, Leeson 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 (Supplementary material Table S5). The N protein of SARS-CoV is also known to up-regulate the expression of the pro-inflammatory 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 (Lei 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 & Sawicki, 1998). Our study predicted that many of the phytochemicals of the Kadha have a significant binding affinity with Nsp3 and Nsp15. Thus, the phytochemicals identified in Table S5 (Supplementary material) may disrupt the formation of RTC and stop the viral genome replication (Supplementary material Table S5).

Since molecular docking provides static poses of the most preferred conformations of a molecule against a target protein, MD simulation studies were carried out. MD simulation studies are helpful in the prediction of several features about a protein-ligand complex such as stability of binding poses gained from docking, calculation of binding energy as well as energy contribution by different residues, and many more. MD simulation employs the classical Newtonian physics and gives information about the actual movement and structural perturbations of a protein in its biological environment. Here, MD simulations were carried out for SARS-CoV-2 Mpro and human ACE2 receptor with selected phytochemicals in the solvated states. RMSD plot analysis shows that the interaction of the phytochemicals with Mpro and ACE2 are stable (Figure 4). RMSF plot analysis also shows a lack of any major fluctuations and the presence of very few moderate fluctuations (Figure 5).

Further, MM-PBSA binding energy calculation was done with the MD output files. The overall binding energy is a cumulative sum of van der Wall, electrostatic, polar solvation and SASA energy. Binding energy calculated using 'g_mmpbsa' package and MmPbSaStat.py and MmPbSaDecomp.py python script for Somniferine A, Tinosporide, Tinocordioside, Orientin and Withanolide against Mpro are −191.8, −96.63, −60.93, −77.38 and −22.82 kJ/mol, respectively. Similarly final binding energy for Somniferine A, Withaferine A, Withanolide, Withanolide B and Vicenin against ACE2 are −1048.11, −72.68, −112.03, −113.26 and 468.4 kJ/mol, respectively. Our findings on binding energy values corroborated positively with the molecular docking study except for the interaction of Vicenin with ACE2 receptor. Contributions of different residues towards the interaction show that phytochemicals Somniferine A has interaction with a large number of amino residues in Mpro and ACE2 proteins as compared to other molecules.

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 ACE2 expressing epithelial cells in the air sacs (alveoli) in the lower lungs. The damaged epithelium leads and interlobular septal thickening lead to leaky cell junctions,



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

accumulation of fluid that is rich in proteins, inflammatory mediators and Pneumonia. The spread of the virus from the lung to the systemic circulation and excessive production of the 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 a significant binding affinity with the many of these inflammatory mediators or the molecules involved in this process (Table 2). It is well known that NF-κB is the master regulator for several genes such as COX-2, VEGF (vascular endothelial growth Factor), proinflammatory 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 signaling pathways. The first pathway involves NIK/IKK whereas the second pathway involves MAPKs and both cause the induction of transcriptional activation of NF-κB. NF-κB-inducing kinase (NIK) is a key mediator of the non-canonical NF-κB signaling 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 migrate to the nucleus and bind to κ elements on DNA in enhancers and promoter regions. Various herbs have the potential to control the inflammation-associated disease by decreasing the production of the proinflammatory 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 2) 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 (Huang et al., 2020). At the same time, SARS-CoV-2 infection also initiates increased secretion of T-helper-2 (Th2) cytokines (e.g. 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 COVID-19 patients.

Our in-silico drug-likeness and pharmacokinetic prediction study show that many of the active phytochemicals found in the Ayurvedic Kadha have reasonably high intestinal absorption (Supplementary material Table S7). Except for berberine, other phytochemicals are negative for AMES test. Some of the phytochemicals such as Berberine, Brachyamide B, Chavicine, Crategolic acid, Jatrorrhizine, Oleanolic acid, Piperettine, Pipericide, Piperolein B, Retrofractamide A, Tinosporide and Ursolic acid were also predicted to be hepatotoxic at higher doses. Thus, consumption of Ayurvedic Kadha would allow absorption of active phytochemicals which can interact with the key viral proteins including spike and main protease, and also human proteins needed for the viral life cycle. This interaction will be helpful in inhibiting virus entry and multiplication in the host cells.

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 a 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 the potential to interact with most of the druggable proteins selected in this study. In conclusion, regular consumption of ayurvedic Kadha in consultation with an 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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Disclosure statement

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