

IN-SILICO INVESTIGATION BASED PLANT SECONDARY METABOLITES AS PROMISING IMMUNOSTIMULANTS AGAINST COVID-19

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Abstract

Objectives: The present study is focused to introduce a better preventive and treatment prospective to fight against the COVID-19 crisis by improving the immune system along with inhibiting the viral activity by a comprehensive review and supportive *in-silico* study. **Methodology:** The study was conducted with a systemic review on the phytochemicals possessing immunostimulatory potentiality, which was further supported by an *in-silico* investigation of those phytochemicals in improving the immunity of the patient as well as in preventive measures which can contribute a major part to manage the dangerous consequences of Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2). **Results:** In *in-silico* study showed that scutellarein, hesperidin and baicalin exhibited strong binding affinity with ACE2, PAK1, Protease by strong molecular interaction forming several hydrogen bonds. **Conclusion:** The present finding interprets scutellarein, hesperidin and baicalin to be potential herbal immune boosters with antiviral potentiality which are to be further explored by *in-vitro* and *in-vivo* methods.

Keywords: Immunostimulants; SARS-CoV-2; COVID-19; terpenoids; flavonoids; ACE2; PAK1; proteases

Introduction

In the present scenario of Corona Virus Disease 2019 (COVID-19) worldwide outbreak suffered most of the countries as well as regions, and developed a major challenge for global health, and seriously affecting the social economy. Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) is a novel beta corona virus primarily communicated between human as droplets infections and personal contact affecting mainly the respiratory system. The second wave of COVID-19 spread shackled India

showing that it is now a crucial time to enhance the management of the disease before its proceeding to the next phase by boosting the personnel immunity through proper vaccination and maintaining the utmost hygiene. The changing variant of Corona Virus Disease 2019 (COVID-19) has forced the search for effective medication against the pandemic developed a scope towards searching potential elements from herbal sources which could help in fighting against the same. The pandemic

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has compelled many countries to close their economic affairs, education and sports to restrict the propagation of the disease. It can be possible by two alternatives; by introducing antivirals or by improving the immune system of the patients as significant therapeutic support. This can be come up with reducing the morbidity along with mortality related to SARS-CoV-2 infections. The clinical consequences of recently arising SARS-CoV-2 are attracting the attention of the scientific and medicinal investigations for using immunostimulants with tremendous success. It is quite clear that immunological targets differ for different types of viruses (Ahmed *et al.*, 2020). The scientific investigations have explored mostly two approaches to manage SARS-CoV-2 infection successfully. The first approach is by prescribing of immune suppressors like corticosteroids to reduce the virus induced hyperinflammation. However, the exact therapeutic window and the etiopathophysiology of corticoids in treating SARS-CoV-2 is not yet clear. On the other hand, corticoids inhibit immunity and hence might obstruct the microbial elimination (Shang *et al.*, 2020; Zhou, *et al.*, 2020). Whereas, the suppressed immunity is found to be a key reason of increased severity of COVID-19 infection (Sharangi, *et al.*, 2021), enhancing patient susceptibility to acquire microbial infections (Otto, *et al.*, 2011). The second approach is by using immunostimulants, such as PRR ligands (Pattern Recognition Receptors), immune checkpoint inhibitors, cytokines, antimicrobial peptides and signaling proteins by eradicating the virus following the stimulation of individual's immunity. It has been believed that a strong immunity is an utmost important aspect of any individual for combating various infectious diseases. In COVID-19 pandemic it is also a boon era for investigation. Immunostimulation is targeted for the benefit of not only the infected patients but also the healthy human being, persons with pre-existing morbidities as well as recovered patients from COVID-19. It can be considered as an overall management of COVID-19. Ayurveda is exceptional with different therapeutic alternative for the management of toxic infections (Goothy, *et al.*, 2020). Based on WHO (World Health Organization), most (3/4th) of people on earth depend on herbal medicines to treat various ailments. Mostly, the contents of the medicinal plants as *Glycyrrhiza glabra*, *Gingiber officinale*, *Allium sativum*, *Citrus lemon*, *Brassica oleracea*, *Mentha piperita* L, *Ocimum sanctum*, *Foeniculum vulgare* Mill, *Thymus vulgaris* L, *Cinnamomum cassia* and *Illicium verum* were ought to be tried in the various structures to frame a compelling

medication for COVID-19 management (Yasmin, *et al.*, 2020; Khanna *et al.*, 2021). The rescheduling for exploration of herbal medicines, provide a noble approach against microbial infections and their transmission. To manage COVID-19 the utilization of herbal origin, that had been employed to manage several epidemic conditions were adopted by many researchers (Shang *et al.*, 2020). In many cases, it has been observed that, there is a lack of direct confirmation regarding the impact of traditional remedies in restoring COVID-19 severity. The conventional as well as home remedies has demonstrated immunostimulatory efficacy which are to be utilized for the prevention measures to encounter the manifestations of COVID-19 (Panda *et al.*, 2020; Mamidi and Gupta, 2021). The decoction of ginger, clove and black piper have been prescribed for COVID-19 patients, as it offers immunostimulatory reactions and brings down the aviation route hypersensitiveness and respiratory manifestations (Bui *et al.*, 2020; Li *et al.*, 2020; Carrasco-Sánchez *et al.*, 2021). As an extensive study emodin, rhein, and chrysin from genus *Rheum* and *Polygonum*; were observed to inhibit the incorporation of S protein of SARS-CoV-2 by binding to ACE (Angiotensin Converting Enzyme) receptor (Ho *et al.*, 2014). Thus, the wellbeing and clinical attributes of conventional medications have been evolved and utilized by several researchers against SARS-CoV-2 (Mohammadi and Shaghghi, 2020). The present study highlights the key target for the immunostimulation to fight against COVID-19 infection which has been supported by *in-silico* study of the major bioactive components of useful medicinal plants for their binding affinity to most promising targets to best manage the recent pandemic. A esteemed bunch of the restorative plants in Ayurvedic Rasayana have been experimentally examined for their potential as well as promising outcomes. A number of bioactive principles from natural source were proved for potential immunostimulatory activity that can be explored for future research. The main objective of this study is to enlist the outcomes of the investigations performed on immunostimulators of herbal sources. Most of the plants have been with their phytoconstituents responsible for immunomodulation have been explained. The study is also targeted to establish herbal bioactive moieties as strong and safe candidates for the successful management of SARS CoV-2 by *in-silico* screening to correlate their mechanism behind immunostimulation. This work will hopefully motivate the investigators to step forward on herbal resources with immunostimulatory potentiality against different viral infections.

Restorative Plants as Antiviral Warriors Against SARS-CoV-2

Especially, natural remedies enrich us with tool kit to prepare novel antiviral items. This practically includes the comprehension of antiviral mode of action of active moieties and their part in bothering infection pathophysiology, survival and its delivery. A broad research is being performed to obtain a potent formulation against coronavirus from herbal origin. The preclinical study on different herbal origins such as *Zingiber officinale*, *Scutellaria baicalensis* Georgi, *Salvia miltiorrhiza* Bunge, *Allium sativum*, *Lecas aspera*, *Abutilon indicum*, *Sphaeranthus indicus*, *Indigofera tinctoria*, *Vitex trifolia*, *Cassia alata*, etc showed significant effectiveness for the management of SARS-CoV infection (Rathinavel *et al.*, 2020; Choudhary *et al.*, 2021). It has also been observed that sinigrin, β -sitosterol, aloe emodin, haesperetin and indigo obtained from *Isatis indigotica* inhibited the SARS-CoV 3CLpro (Lin *et al.*, 2005; Wen *et al.*, 2007; Fuzimoto and Isidoro, 2020) observed that lignoids and abietane type diterpenoids have best anti-viral effects out of 221 phytoconstituent evaluated after isolating from *Juniperus formosana*, *Chamaecyparis obtuse* and *Cryptomeria japonica* and so from *Gentia scabra* and *Cassia tora* as anti-SARS-CoV potentiality (Wen *et al.*, 2007; Natalie, *et al.*, 2021). Caffeic acid, connamic acid, chalcones, betulinic acid and curcumin obtained from herbal origins are confirmed by various researchers to have potent inhibitory potentiality of SARS-CoV 3CLpro protease. Tetra-O-galloyl beta-d-glucose from *Galla Chinensis* has been observed to exhibit extensive binding affinity towards SARS-CoV spike (S) protein (Valerie, *et al.*, 2008; Abiri *et al.*, 2021; Bhattacharya *et al.*, 2021). Additionally, lectins from *Nicotiana tabacum*, *Nicotiana benthamiana*, and *Urtica dioica* exhibited inhibitory potentiality towards SARS-CoV proliferation (Keyaerts *et al.*, 2007, Zheng *et al.*, 2020, Demurtas *et al.*, 2016). Potential SARS-CoV PLpro (Papain Like Protease) inhibitors like tomentins from *Paulownia tomentosa* was confirmed by Cho *et al.*, 2013 and chalcones *Angelica keiskei* was proved by Park *et al.*, 2012. Jo *et al.*, (2019) also observed herbacetin of *Litchi chinensis* and *Linum usitatissimum* to possess inhibitory effect against 3CLpro (Crystal structure of native SARS) in MERS-CoV. *C. tematea* was also observed to be MT (Metalloprotease) inhibitor, ADAM17 (A Desintegrin and Metalloproteinase domain 17), associated with munching ACE-2 enzyme, which is playing key role in virus replication (Maity *et al.*, 2012; Vellingiri *et al.*, 2020; Zipeto *et al.*, 2020). Viral RNA (Ribonucleic acid) synthesis was observed to be

blocked by *Strobilanthes cusia* which was modulated papain like proteases potentiality objecting HCoV (Saxena, 2020; Tsai *et al.*, 2020; Jha *et al.*, 2021). Subsequently, different medicinal plants exhibited potentiality against ACE like *Cassia occidentalis*, *Punica granatum*, *Boerhaavia diffusa*, etc (Hussain *et al.*, 2018; Khan and Kumar, 2019). Presently, Liu *et al.*, (2020a), *A. paniculate* was proved to repress NOD (Nucleotide-binding Oligomerization Domain)-like receptor proteins (NLRP3), IL-1 beta (Interleukin-1 β), C-1 (Caspase-1) molecules, mostly involved with SARS-CoV pathophysiology. In association with these, *Lycoris radiata*, *Isatis indigotica* have, *Lindera aggregate* and *Artemisia annua* exhibited anti-SARS-CoV potentiality (Nair, 2012; Mishra *et al.*, 2014; Rege and Chowdhary, 2014; Ganjhu *et al.*, 2015; Thayil and Thyagarajan, 2016). Interestingly, the potentiality of *Osimum sanctum* has been screened for its potentiality against COVID-19 (Goothy *et al.*, 2020). Simultaneously the important bioactive dipeptide; Aurantiamide acetate of Piper along with EK1 (Eph-like Kinase1) are observed scientifically as potential inhibition of SARS-CoV, thereby can effectively be indicated to combat the COVID-19 crisis. Some major promising and imperative bioactive phytoconstituents can be employed for the mitigation of the raised emergency of managing COVID-19. Figure 1 elaborates the potential possible targets for the herbal resources as suggested by Wruck and Adjaye, 2020 and Table 1 enlists the herbal resources with their potentiality against viral infection.

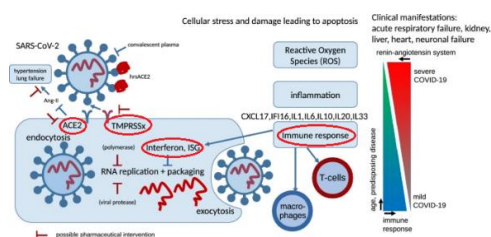


Figure 1. It shows pathophysiology of SARS-CoV-2 invading and proliferation in human host (Represented by Wruck and Adjaye, 2020). It shows the binding of SARS-CoV2 to ACE2 receptor at the host cell membrane and the transmembrane serine protease TMPRSS2 mediates the viral entry. Hence these can be targeted to inhibit the viral attack. The replication of viral RNA can be inhibited by the interferons and ISG (Interferon Stimulating Genes). The packed virus gets released from the infected cell to attack macrophages, T-Cells, etc. due to the disturbed immune system which clearly indicates the immunostimulators as the best management for SARS-CoV-2 infection

Table 1. It shows Potential targets for immunostimulation in COVID-19 infection

Name of the target	Mechanism	Resultant clinical manifestations	References
ACE2 receptors	After binding to ACE2, SARS-CoV-2 liberates its RNA and replication starts.	It potentiates the liberation and accommodation of ACE2 with virus RNA and gradual decrease in ACE2 levels. It causes a disturbed ACE2 and ACE arms of RAS (Renin–Angiotensin System). It leads to decreased Ang1-7 activity and enhanced AT1 (Angiotensin1) activity resulting inflammatory responses. It also enhances cytokine resulting in multiorgan damage along with thrombosis.	Goyal <i>et al.</i> (2020) Salamanna <i>et al.</i> (2020) Ferrari <i>et al.</i> (2021) Srivastava <i>et al.</i> (2020).
ACTH (Adrenocorticotrophic Hormone)	Activation of ACTH	Increase in adrenaline, non-adrenaline and glucocorticoids enhancing the pathogenicity by causing immunosuppression by impaired immune mediators like NK (Natural Killer) cells, Helper-T-cells, lymphocytes, etc.	Calder <i>et al.</i> (2020)
PAK-1 (P21-Activated Kinase 1) targeting RAC/CDC42 (Cell Division Control Protein 42) activated kinases	It inhibits the LLC2 linked fibrosis due to COVID-19 infection.	Due to the correlation of LLC2 with ACE2, binding of SARS-CoV to receptor CK2/RAS-PAK1-AP1 was found to suppress host immunity.	Lu (2020) Chen <i>et al.</i> (2018)
COVID-19 proteases	Its activation causes the polyprotein fragmentation	By polyprotein fragmentation it causes decreased immunity.	Tsai <i>et al.</i> (2020) Chen <i>et al.</i> (2020) Zhang <i>et al.</i> (2020)
S-IgG (Immunoglobulin G)	Monocytes/macrophages	It showed enhanced infiltration of monocytes/macrophages in the lungs along with overturning the macrophage at the onset of the infection.	Florindo <i>et al.</i> (2020)
PD-1 (Programmed Death-1) and PD-L1 (Programmed Death Ligand-1)	T-Helper cells	T-cell depletion in septic patients 160.	Zhang <i>et al.</i> (2004)

Bioactive Moieties as Immunostimulants for COVID-19 Infection

Terpenoids

Terpenoids show the modification in cell-based modulation of cellular biotransformation, mainly steroidal biosynthesis along with the screened antiviral potentiality (Malinowska *et al.*, 2013). After invading the host cell, RNA virus induces the formation of two polyproteins, helps in regulation of RNA synthesis, and activation of protease enzymes. and also associated with polyprotein fragmentation (John *et al.*, 2015; Cui *et al.*, 2019). Molecular dynamic simulations have shown the potentiality of herbal constituents against viral infection (Table 2).

Polyphenols/flavonoids

From the literature review it was confirmed that, among the phytoconstituents, polyphenols show a wider range of therapeutic potential. They used to enhance the immune response of the cells against microbial infection by acting on various receptor-based signal transduction (Ding *et al.*, 2018). Gradually, more than 5000 flavonoids were identified and elaborated as antiviral compounds against SARS-CoV (Ververidis *et al.*, 2007, Solnier and Fladerer, 2020). Inhibition of cellular receptor kinases such

as MAPKs (Mitogen-activated protein kinases) (Villa *et al.*, 2017). Organosulfur compounds of the *Allium cepa* (onions) and *Allium sativum* (garlic) are observed to have anti-SARS-CoV-2 potentiality (Thuy *et al.*, 2020). Additionally, Sampangi-Ramaiah *et al.* (2020) observed the binding affinity of essential oil of *Coriandrum sativum*, ursolic acid of thyme, rosmarinic acid of rosemary and glucobrassicin of mustard green to 6LU7 and 6Y2E proteases. Table 2 elucidates immunostimulatory polyphenols/flavonoids confirmed by different researchers against COVID-19. Along with which, studies showed etragol, piperol, betlol, eugenol, caryophyllene, etc. were to be responsible for developing immunity within the viral infected persons (Sengupta, 2019). However, researchers have depicted that some herbal formulation containing cinnamon, licorice and selenium against the viral noxious effects by disrupting their entry, replication, proliferation along with the host immunostimulation, thereby, proving evident anti-SARS-CoV-2 potentiality (Polansky and Lori, 2020).

Polysaccharides

The polysaccharides are always explored for their potential therapeutic values against various disorders. Since long back some polysaccharides obtained from the natural sources were screened scientifically and proved to possess significant

Table 2. It shows bioactive moieties from herbal sources against viral infections

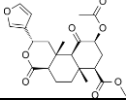
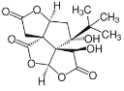
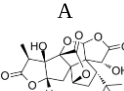
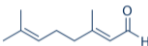
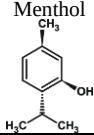
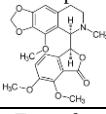
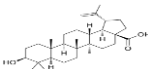
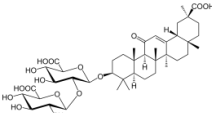
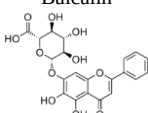
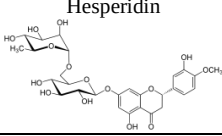
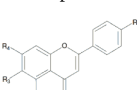
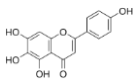
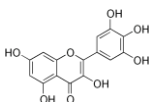
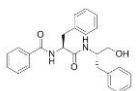
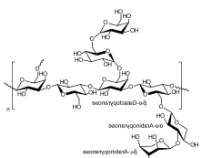
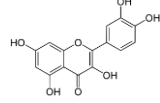
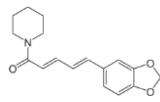
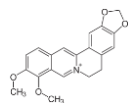
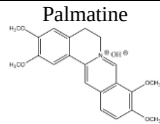
Plant name	Bioactive moiety	Mode of Immunostimulation	References
Terpenoids			
<i>Nigella sativa</i>	Thymoquinone 	It interacts with the SARS-CoV-2 spike protein to the HSPA5 SDBb (substrate-binding domain b) of SARS-CoV-2 and depresses the infection.	Ahmad <i>et al.</i> (2020)
<i>Salvia divinorum</i>	Salvinorin A 	By inhibiting the activity of viral protease these bioactive phytoconstituents suppress the viral infection.	
<i>Ginkgo biloba</i>	1. Bilobalide  2. Ginkgolide A 	By modulating MAPK activity, mitochondrial membrane and DNA fragmentation it initiates apoptosis.	Elfiky (2020)
<i>Backhousia citriodora</i>	Citral 		
<i>Mentha piperita</i>	Menthol 		
<i>Papaveraceae</i> family	Noscapine 		
<i>Plectranthus barbatus</i>	Forscolin 		
<i>Apium graveolens</i>	Beta Selinene 		
<i>Betula alba</i> var	Betulinic acid 		
Polyphenols/flavonoids			
<i>Glycyrrhiza glabra</i>	Glycyrrhizin 	Due to its anti-inflammatory and antioxidant activity, the generation of interferons contributes its effectiveness against SARS-CoV infection. Additionally, it inhibits the viral ribosome along with viral protein translation and synthesis.	Ramos-Tovar <i>et al.</i> (2020) Pilcher (2003) Yeh <i>et al.</i> (2013) Mohammadi and Shaghaghi, (2020) Nourazarian <i>et al.</i> (2015) Keyaerts <i>et al.</i> (2007)
<i>Scutellaria</i> genus	Baicalin 	Its potential antioxidative and antiapoptotic activity was profound in pulmonary arterial hypertension. It is supported by additional findings of its effect on ACE2 enzyme.	Liu <i>et al.</i> (2020a)
Citrus peel	Hesperidin 	It has potential affinity to most of the targets of COVID-19 starting from viral entry, to viral genome transcription and replication.	Meneguzzo <i>et al.</i> (2020)
Bees	Propolis 	It has been observed to inhibit PAK1 by the down-regulation of RAC and so effective in blocking COVID/coronavirus curbed respiratory fibrosis.	Nguyen <i>et al.</i> , 2017 Maruta, 2020

Table 2. (Continued)

Plant name	Bioactive moiety	Mode of Immunostimulation	References
Polyphenols/flavonoids			
Isatisindigotica	Scutellarein 	It causes inhibition of SARS-CoV enzymes like RNA polymerase and 3CL-proteases.	Li <i>et al.</i> (2005) Yu <i>et al.</i> (2012)
Torreya nucifera	Myricetin 		
Piper aurantiacum	Aurantiamide 	It is effective against COVID-19 infection by its chymotrypsin like protease activity.	Joshi <i>et al.</i> (2018) Srivastava <i>et al.</i> (2020)
Polysaccharides			
Guduchi or Giloy herb	Arabinogalactan polysaccharide 	It has been observed to possess increased serum IgG level along with the stimulation of macrophages. In another finding it induced the cell regulated immunity along with the overall humoral immunity and so inducing adaptivity.	Subhose <i>et al.</i> (2005) Winston and Maimes (2007)
Camellia sinensis	Quercetin 	It has been observed to induce immune power along with the inhibition of viral entry, replication, proteases and infection.	Polansky and Lori (2020) Massi <i>et al.</i> (2017)
Alkaloids			
Piper longum Linn	Piperine 	It effects of T-cell as well as B-cell phenotypes with cytokine secretion. It also modulates the macrophages modifications by oxidative stress with programmed cell death, lymphocyte phenotyping, cell proliferation and cytokine release. It has been supported by its inhibitory effect on TGF- β (Transforming Growth Factor- β) signaling pathway in human lungs.	Pathak and Khandelwal (2009) Fonseca <i>et al.</i> (2020)
Berberis aristata	Berberine 	It enhances the interleukin-12 production, causing immunostimulation by reducing T helper-2 cytokine in CD4+ cells. It inhibits Th17 (T-helper 17) activity and CD40, CD80 and CD86 expression expression of CD40, CD80, and CD86) and IL-6, IL-1b, IL-23 discharge.	Yang <i>et al.</i> (2013) Chabib <i>et al.</i> (2018) Wang <i>et al.</i> (2021)
Tinospora cordifolia	Palmitine 	It modifies the level of antioxidants, enhances T and B-cells, antibody, cytokine concentration resulting potential immunomodulation.	George <i>et al.</i> (2016) Majnooni <i>et al.</i> (2021)

immunostimulatory activity (Martinez *et al.*, 2005). Pujol *et al.* (2002) identified different sulfated polysaccharides from marine algaewhich were found to be effective against the viral infections in human as well as animals. Nagle *et al.* (2020) proposed

effective antivirals like chondroitin sulfate and heparin; obtained from marine red green growth (Ghosh *et al.*, 2009 ; Seedeve *et al.*, 2013). In addition to this, the exo-polysaccharides from Porphyrite were believed to be having a strong effect against

retrovirus, etc (Talyshinsky *et al.*, 2002; Nagle *et al.*, 2020). Henceforth, the polysachharides from the medicinal plants can be utilized for the management of COVID-19 (Table 2).

Alkaloids

Alkaloids are scientifically proven to possess important pharmacological effects like anti-inflammatory, anticancer activities, etc. Along with all these literatures showed the evidence of significant immunostimulatory activity of several alkaloidal principles from herbal origin. Table 2 elaborates the bioactive principles of alkaloid origin from different herbal sources with prominent immunostimulatory effects.

In-silico Investigation of Potent Immunostimulant for COVID-19

During all sorts of crisis in the present situation of COVID-19 pandemic as well as latest online tools for drug evaluation, molecular docking of bioactive moieties has been proved to possess potentiality to manage this dangerous infection targeting the strengthening of the immune power of individuals. Rolta *et al.* (2020) adopted molecular docking methodology and evaluated 100 important phytoconstituents of drumstick, garlic, Himalayan rhubarb, Leafy Meadow-Rue, nutmeg, Tejbai and Punarnava for potentiality in binding to RNA such as NTD (N-terminal Domain) and PDB 6VYO (Protein Data Bank in Europe) of nucleocapsid phosphoproteins of SARS-CoV-2 of nucleocapsid phosphoprotein of SARS-CoV-2. Similarly following *in-silico* study, Rolta *et al.* (2020) confirmed the potent NTD of RNA binding affinity of emodin, alizarine, anthrurufin from Aloe emodin and dantron from Rheum emodin. Adem *et al.* (2020) had also investigated the herbal polyphenols for protease (Mpro) inhibition potentiality following *in-silico* method against SARS-CoV-2 using flavonoids. The study showed the efficacy of rutin, apiin, hesperidin, diosmin and diacetylcurcumin towards inhibition of protease enzyme, which leads to decrease the infection rate of the corona virus. Recently, organosulfur materials like allyl disulfide and allyl trisulfide of Allium in an *in-silico* study, the organosulfur materials such as allyl disulfide and allyl trisulfide from garlic proved to possess efficient binding to ACE2 significantly and targeted for the treatment of SARS-CoV-2 (Thuy *et al.*, 2020). Continuing the process of scientific contribution to provide an effective immunostimulant along with inhibiting the viral activity to prepare in a better manner for the fight to COVID-19, *in-silico* procedure is followed to explore some important bioactive moieties from herbal origin.

Materials and Methods

Target Preparation, Active Site identification and selection of Grid Center

Based on the resolution and full-length sequence coverage including RBD (Receptor Binding Domain) region of ACE2, PAK1, Protease, PD1, PD-L1 all the 3D X-ray Crystallographic structures were observed from RCSB-PDB (Protein Data Bank). Removal of water molecules, ions and covalent ligand was done by using Dockprep procedure implemented in the UCSF Chimera program. Kolmann charges were computed, polar hydrogen atoms were subsequently added. The active site and list of key amino acid residues interacting with ligands of such macro molecules was taken from Literature.

Molecular Docking with Ligand Preparation

Molecular Docking and measurements were accomplished Auto Dock 4.2. This allowed for consistency checking and increased the confidence in the results. The Active sites were taken from literature and the Center of the Grid Box for each individual molecules were calculated based key amino acids at active site and the optimal box size was calculated by eBoxSize Script developed by Feinstein *et al.* (Feinstein *et al.*, 2015). For Auto Dock 4.2, Lamarckian Genetic Algorithm was used with 1,000 runs in Auto Dock 4.2. The best docked pose was selected centered upon their binding energy score and its binding energy score and significant binding to active sites. In the present study, the 3D (3-dimensional) structures of the molecular ligands were obtained from Pubchem Database. These structures were checked for any errors. Then by using Open Babel (Boyle *et al.*, 2021) Hydrogen was added and the final shapes were modified into PDBQT (Protein Data Bank, Partial Charge (Q) and Atom Type (T)) setup of Docking observations. A detailed description of all the Ligands and its SMILE, Molecular Formula, Molecular weight, cLogP has presented in Table 3.

Results and Discussions

A detailed description of all the targets and its sequence length, Ref-Seq id, Active sites, Grid Center has presented in Table 3. Molecular level Molecular docking studies is the accurate prediction of protein-ligand interaction geometries. The docking was performed to obtain the best orientation and affinity (hydrophilic and hydrophobic interaction) of each and every moiety at the targeted potential binding site of the proteins. Which ranges

Table 3. It shows *In-silico* study on the ligand targets for better immunostimulation

Drug Targets	Sequence Length	NCBI Reference Sequence ID	PDB ID	List of interacting key amino acid residues Present in Active Site	Grid Center	References
ACE2	805	NP_068576.1	6m0j	SER19, ILE21, GLN24, THR27, PHE28, ASP30, LYS31, GLU35, GLU37, ASP38, TYR41, GLN42, LEU45, LEU 49, MET82, TYR 83, THR324, GLN3	X = -33.24555 Y = 28.49164 Z = -2.779875	
PAK1	486	NP_001363224.1	3FXZ	ILE276, Val284, ALA297, VAL328, GLU345, MET344, TYR346, LEU347, ASN394, LEU396	X = -11.137894 Y = 34.860000 Z = - 11.887471	Maksimowska <i>et al.</i> (2008) Singh <i>et al.</i> (2021)
Main protease (Mpro), also known as 3CLPro	306	YP_009742612.1	6Y2E	SER81, CYS145, HIS163, GLU166, GLN189, HIS164, PHE140, GLY143	X= -15.4 Y= -27.247 Z= 1.186	Zhang <i>et al.</i> (2020)
PD1	288	NP_005009	6UMT	VAL64, ASN66, TYR68, MET70, SER73, ASN74, GLN75, THR76, ASP77, LUS78, ALA80,ALA81, SER83, GLU84, GLU88, VAL90, LEU122, GLY124, ILE126, SER127, LEU128, ALA129, PRO130, LYS131, ALA132, GLN133, ILE134, GLU136	X= -10.601385 Y= -6.482887 Z= 25.172854	Lee <i>et al.</i> (2016)
PD-L1	176	NP_001254635	5O45	ALA121, ARG113, ASN63, ASP122, GLU58, MET115, TYR123, TYR56, GLN66, ILE54, SER117, VAL68, ASP61, GLU60, ARG125, GLY120, LYS62	X= 22.491321 Y= 5.024970 Z= 15.873355	Liu <i>et al.</i> (2019)

ALA: Alanine; ARG: Arginine; ASN: Asparagine; ASP: Aspartic Acid; CYS: Cysteine; GLU: Glutamic acid; GLY: Glycine; HIS: Histidine; ILE: Isoleucine; LEU: Leucine; LYS: Lysine; MET: Methionine; PHE: Phenylalanine; PRO: Proline.

from -11.07 kcal/mol to -7.67 kcal/mol. The interaction results of calculated binding energy of all 13 compounds are listed in Figure. 2. In this study, it was found that scutellarein, hesperidin and baicalin are showing strong binding affinity with ACE2, PAK1, Protease along with strong molecular interaction by forming several hydrogen bonds (Figures 3, 4, 5, and 6). Hydrogen bond stabilizes the ligand at the active site. It has also been found that these molecules also have good physiochemical properties like molecular weight and logP.

Conclusions

A systemic approach has been made to enlist the potential bioactive phytoconstituents in the management of COVID-19 pandemic in an effective and less toxic manner. The herbal origins are popular in their use due to less secondary untoward effects. The target of the research in medical science is now focused towards to obtain a promising treatment for combating the changing variants of SARS CoV-2. In the present scenario, the review along with the *in-silico* approach of promising phytochemicals by boosting up the immune power of individual will be helpful in managing the worsen situation of COVID-19. The study reported here

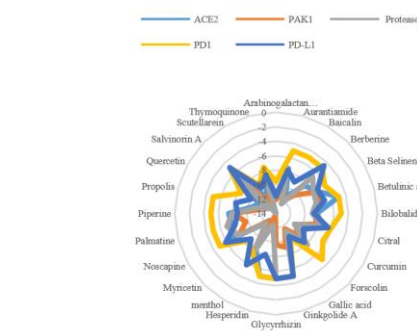


Figure 2. It shows receptor Ligand Interaction of selected bioactive herbal constituents

scientifically proven herbal bioactive moieties to a single stage as alternative drug therapeutics and confirmed by *in-silico* study which act against different selected targets of immunostimulation which may be some targeted and potent therapeutic candidates against COVID-19 infection facilitating the more intense scientific investigations to overcome COVID-19 crisis of the world and the COVID-19 disaster in India due to its horrific waves at present and in future. Therefore, these means can reboot the resistant framework and strengthen the inward powers to fight against extreme viral diseases COVID-19 separately. The present study

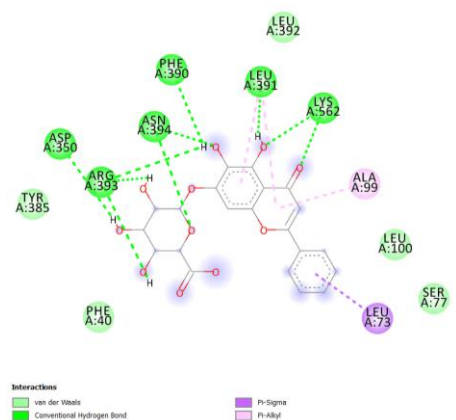


Figure3. It shows Interaction of beclinal to ACE2

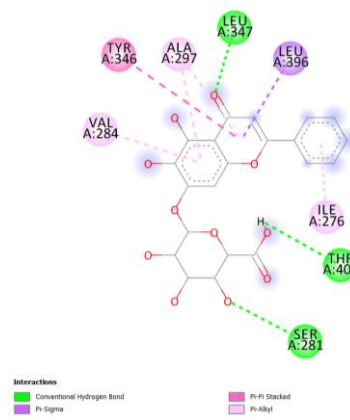


Figure. 5. It shows interaction of baicalin with PAK1

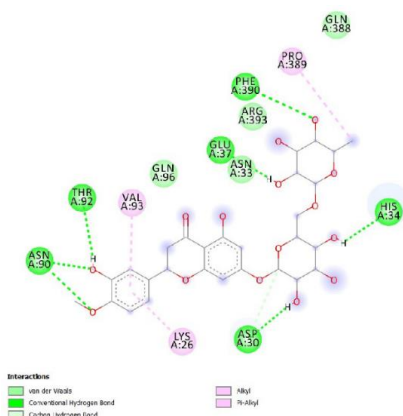


Figure 4. It shows interaction of hesperidin to ACE2

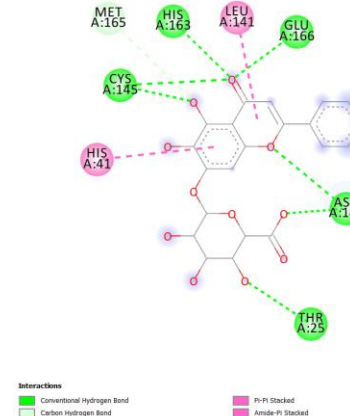


Figure. 6. It shows interaction of baicalin to Protease

has elaborated the promising targets for immunostimulation and reduced viral activity byscutellarein, hesperidin and baicalin which showed strong binding affinity with ACE2, PAK1, proteases with strong molecular interaction by forming several hydrogen bonds along with good physiochemical properties like Molecular Weight and logP. Henceforth these molecular can be taken for further preclinical studies to validate the Insilco findings. Scutellarein, hesperidin and baicalin could be taken for further in-vitro and in-vivo studies to validate the *in-silico* findings to curb the research more focused to scrutinize their effects on COVID-19 focusing reduced viral activity along with the immunostimulatory potentiality. The contents are also equally supportive in the field of research along with the industries to find out and screen promising herbal sources for development of herbal antiviral agent along with immune boosters. The present findings will be beneficial to invest in healthcare for the better management and mitigation of COVID-19 with a glory of defeating successfully.

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