In silico Analysis of ACE2 Receptor to Find Potential Herbal Drugs in COVID-19 Associated Neurological Dysfunctions

Juan Hou¹, Adil Manzoor Bhat², Shaban Ahmad², Khalid Raza², and Sahar Qazi²

Abstract
COVID-19 mainly causes the collapse of the pulmonary system thereby causing a dearth of oxygen in the human body. Patients infected with this viral disease have been reported to experience various signs and symptoms associated with brain dysfunction, from the feeling of vagueness to loss of smell and taste to severe strokes. These neurological problems have been reported by younger COVID-19 infected patients mainly in their thirties and forties. Various researchers from around the globe have discerned numerous other brain dysfunctions, such as headache, dizziness, numbness, major depressive disorder, anosmia, encephalitis, febrile seizures, and Guillain-Barre syndrome. The involvement of the CNS by this viral infection has been predicted to be for a longer period of time, even if the patient recovers from COVID-19. The neuronal cell damage caused by COVID-19 is a potent factor responsible for cognitive, behavioral, and psychological problems among its sufferers. The hypoxic conditions can also trigger the formation of beta-amyloid plaques and tau-tangles and thus the virus can even induce Alzheimer’s in patients in the near future. The virus affects the brain directly, thereby causing encephalitis. This pandemic has also been shown to have a negative psychological toll on people. This research aims to highlight the brain dysfunction associated with the ACE2 receptor that is known to be a crucial player in the COVID-19 pandemic using genetic networking approaches. Furthermore, we have identified herbal drug candidates that bind to the ACE2 receptor in order to identify potential treatments for the neurological manifestations of COVID-19.

Keywords
ACE2, COVID-19, molecular docking, molecular dynamic simulation, network analysis, phytochemicals

Received: April 29th, 2022; Accepted: July 22nd, 2022.

Introduction
COVID-19 is a non-biotype viral infection that is transmitted in humans of all age groups and is responsible for causing serious respiratory tract infections, in severe cases, even leading to death¹. It originated initially in Wuhan city of China in 2019 where it was discerned to be of Beta-BAT-SARS-CoV-2 lineage; however, it has become a source of distress globally since then². Various online social platforms and the global research fraternity have produced humongous data in myriad aspects namely—social, technological, global news, healthcare, political domain, vaccination discovery, and drive, helping to understand the myriad aspects of the ongoing pandemic. Quite recently, many researchers have found that COVID-19 has negative implications on various neurological disorders. Different strains of coronaviruses have been shown to cause neurotropism and neuro-invasive symptoms leading to various brain dysfunctions in COVID-19 affected populations³.

SARS-CoV-2 belongs to the Nidovirales order. Common features of the viruses belonging to this category are—(i) conserved and highly organized genomic contents - the replicate gene comes first, followed by the structural and accessory genes; (ii) non-structural gene expression that is caused by ribosomal framesshifting; (iii) enzyme dynamics taking place within the replicate-transcriptase polyprotein; and (iv) 3′-subgenomic mRNA formation resulting in downstream gene expression³. Until last year, COVID-19 was thought to be fatal for elderly people or for individuals with co-morbidities. However, in early
2021, various strains were identified that were tagged to be catastrophic for younger people and infants too. As per the CDC report, there are mainly 5 variants of concern (VoC) of COVID-19 that cause severe damage namely - a) B.1.1.7 (initially detected in South Africa, but severely affected the US in early 2021), b) B.1.351 (affected the US in early 2021, but was first rectified in South Africa), c) P.1 (was first observed in travellers from Brazil who tested positive in initial COVID-19 screening in Japan; this variant also caused severe damage in the US population, as reported in January 2021), d) B.1.427 and B.1.429 (these were first identified in California in February 2021 and were classed as a variant of concern (VoC) in March)\(^5\).

Several researchers have worked hard to identify potential drugs that can treat COVID-19 and related maladies. Today, we have many verified synthetic drug candidates such as riboflavin, lopinavir, oseltamivir, lopinavir/ritonavir\(^6\), minocycline, tocilizumab, ribavirin, and nicosamide\(^7\), 8. Moreover, many natural drug candidates have also been utilized to treat COVID-19\(^8\)-\(^10\). However, there are no herbal compounds that can help in curing the neurological manifestations of COVID-19. Several recently published studies have revealed that after getting infected with SARS-CoV-2, various different neurological dysfunctions, such as stroke, brain stem impairments, neuroinflammation, brain fog, and delirium were observed in patients\(^11\). SARS-CoV-2 has also been revealed to deploy angiotensin-converting enzyme 2 (ACE2) as an entry point to the cells. This simply means that SARS-CoV-2 binds to human ACE2 for entry and attachment inside the host cells\(^12\). The expression of ACE2 in human neurons suggests the neuro-encroaching strength of SARS-CoV-2, which further reassures that the infected patient’s neuronal system could affect the overall respiratory function\(^13\). Additionally, ACE2 is known to be the receptor for SARS-CoV-1 as well as that for SARS-CoV-2. Reports also discern that ACE2 is expressed in neural cells which in turn allows SARS-CoV-1 to cause brain dysfunctions in humans\(^14\).

This research work aims to highlight the neurological dysfunctions that are associated with the ACE2 receptor active in COVID-19. Also, using epiniformatics-based network analysis, we have identified the direct network associates of ACE2, and thus have established why ACE2 is responsible for COVID-19 related neurological manifestations in individuals. By deploying a gene-drug mapping approach, we have highlighted a few synthetic and alternative herbal drug candidates that can be seen as possible treatment strategies for treating the brain dysfunctions associated with COVID-19. Additionally, we executed pharmacological screening, molecular docking, and essential electrostatics analysis on the best suitable phytochemicals to validate their usefulness as a therapeutic and stable affinity with the ACE2 receptor.

### Methods & Methodology

#### Retrieval of Sequence

We used the Angiotensin-converting enzyme 2 (ACE2) which has been discerned to be the entry receptor for SARS-CoV-2 from the National Center of Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/), (accession number NM_001371415.1 and Gene ID: 59272). The protein sequence of ACE2 was also retrieved (accession ID NP_001358344.1) from Protein Data Bank (PDB Id: 7RPV). This is the crystal structure of the affinity-enhancing and catalytically inactive ACE2 in complex with SARS-CoV-2 RBD, respectively. The ACE2 receptor was separated from the complex of spike protein (S1), 2-acetamido-2-deoxy-beta-D-glucopyranose, and attached zinc ion using Pymol.

#### Motif Identification, Enrichment & Annotation Analysis

This was executed using CTCFBSDB software\(^15\), 16, while for enrichment analysis, we deployed the MEME suite’s CentriMo software\(^17\) and comparison TomTom software, available in MEME suite\(^18\). For genomic annotation purposes, we used Rapid Annotation using Subsystem Technology (RAST) software\(^19\) and curated a pipeline that can screen for seed genes and essential genomic annotations for ACE2 transcript files (NCBI Gene ID: 59272). We used the updated annotation scheme named RASTK with call features repeat region SEED minimum identity as 95 and a minimum length of 100; CDS-glimmer\(^3\) was used for a minimum training length of 2000. Along with this, we used the kmer dataset release\(^70\) for annotation purposes.

#### Genetic Network Analysis

The ACE2 protein sequence (NP_001358344.1) was deployed to the WebMGA\(^20\) web server to identify the cluster of orthologs to annotate its function. The network interactors were identified using STRING software\(^21\) by tuning the network parameter settings for displaying only the physical network which has a good confidence score taken from experimentally identified sources, literature, and text mining approaches. The network was classified using the k-means clustering algorithm.

#### Gene-Drug Mapping

We used DGIdb\(^22\), DrugBank\(^23\), and PubChem\(^24\) to explore the potential drug candidates that can serve the purpose of treating COVID-19. The initial criteria to search for the drugs were (a) having a strong interaction with the target seed genes, (b) interaction score should be \(\geq 5.0\), and (c) the identified drugs must be reported in the literature. We executed it in a keyword-based search strategy method, where identified seed genes were separately searched in the databases.

#### Identification of Alternative Herbal Compounds as Potential Drug Candidates

An extensive literature search was executed using PubMed (https://www.ncbi.nlm.nih.gov/pmc/) to identify alternative herbal compounds as potential drug candidates.
Pharmacological Screening Using ADMET

The identified phytochemicals were retrieved from ChEMBL and stored in SMILES format (Simplified molecular-input line-entry system). SwissAMDE webserver was used to assess the phytochemicals for their physicochemical, pharmacokinetic (PK), pharmacodynamic (PD), medicinal chemistry, solubility, and lipophilicity properties.

Molecular Docking

Binding Site Identification. PrankWeb was used to identify binding pockets within the target ACE2 receptor.

Preparation of Target and Ligand Files. PyRx software was used for virtual screening and docking purposes. Using the software, polar charges were added to the target receptor ACE2 and were checked for any additional co-factors/metal/ions bound with the receptor. If any, they were removed and then converted from .mol/.sdf to .pdbqt files. Similarly, the ligands were first energy minimized by conjugant gradient optimization algorithm with the universal forcefield in the background. After minimization, the ligands were saved from the .mol/.sdf to .pdbqt files. Multi-drug docking took place with the grid box set at 60 Å × 60 Å × 60 Å and a spacing of 0.375 Å. The total number of steps was set as 200 with stopping criteria of energy difference less than 0.1.

Electrostatics Computation

Molecular mechanics generalized Born surface area (MM-GBSA) was used to compute the binding free energy (delta G) using the adaptive Poisson-Boltzmann solver (APBS) plugin available in PyMOL software. Moreover, other electrostatic features were also calculated using Blueses software and macromolecular energy landscape was computed using the Frustratometer webserver.

Results

Motif Identification & Enrichment

Myriad motifs have been already identified in the past year in the Wuhan-based SARS-CoV-2 genome. However, we aimed at identifying the motifs that were present in the main entry receptor ACE2 that has been identified to play a direct role in neurological dysfunction caused by SARS-CoV-2. Therefore, we used CTCFBsDB software that works on the CCCTC-binding factor (CTCF), a highly conserved transcription regulator that is found in all organisms ranging from Drosophila melanogaster to Homo sapiens and is said to associate itself with DNA sequences using the 11-zinc fingers. These binding fingers have been said to play a pivotal role in the adaptive divergence of DNA sequences. Researchers have reported these factors to be associated with myriad epigenetic mechanisms such as genomic imprinting and X-chromosome inactivation.

We deployed the ACE2 receptor mRNA sequence (accession Id—NM_001371415.1) to CTCFBsDB and identified six major motifs. Table 1 displays the detailed results of the motif identification using the CTCFBsDB software. Only two motifs were found to be significant, namely motifs M2 and M5, based on their high scores. Motif M2 (AGTACTGCC, score = 10.6676) is present on the negative strand and has a length of 9 nucleotides, while motif M5 (AGTACTG TAGCATGGTGCTCA, score = 11.8863) is also located on the negative strand and has a length of 20 nucleotides. Since these two motifs had higher scores, we proceeded with our motif enrichment analysis using these only. After enrichment analysis, we discerned that the two motifs- M2 and M5 are highly conserved in the mouse genome and there are about 579 non-redundant motifs that are present matching our identified motifs in the JASPAR database, while 386 similar matches were observed in the Uniprobe mouse database. Also, A-T nucleotide content was found to be 0.2908 while C-G was 0.2092.

For comparing the two motifs M2 and M5, we subjected them to MEME suite’s TomTom server that predicted two different matches for each of the two motifs. Table 2 represents the detailed results of the motif comparisons.

It was observed that these compared motifs were matched to zinc fingers, DNA-binding domains, heart and neural crest derivatives, RNA-methyltransferases, and paired like homeoboxes present in the human, mouse, and yeast genome.

The genomic annotation revealed some essential characteristics and information such as the closest neighbours, GC content that directly affects the genome functioning, and species ecology of the ACE2 gene. In total, 280 characteristic features were identified in the ACE2 gene. The GC content was observed to be 38.5% displaying the quadratic relationship with the genomic size of the ACE2 gene. The analogy is simple - the larger the genomic size, the lower the GC content viz., because of the higher biochemical expenditure of GC base formation. There were 15 out of the total 280 characteristic features that were present in the ACE2 gene present mainly in the coding sequence regions. Table 3 encapsulates the detailed report of the 15 best features retrieved, as identified by our genomic annotation.

Network Analysis

We employed the protein sequence of ACE2 (NP_001358344.1) for network analysis. Our main objective was to identify the cluster of orthologs and accordingly interpret the functional annotation of the ACE2 receptor. We identified 25 clusters of orthologs (COGs) for our ACE2 protein that play important biological roles in various cellular processes such as - cell cycle regulation; translation; RNA processing and modification; chromatin structure and dynamics; post-translational modifications (PTMs); intracellular trafficking, secretion, and vesicular control. However, only one COG was found to have a good coverage score of 0.85 with a good abundance score of 0.99 named - Oligoendopeptidase F (COG1164).
The ACE2 protein was further subjected to the network analysis in order to identify its associated interacting partners. There were 10 significant interacting partners of our query protein ACE2, namely AQPEP, ST3GAL4, DENR, NPEPPS, AGTRAP, ITPKB, SOCS2, REN, GHRHR, and SLC15A1. Fig. 1 shows the interacting network of ACE2 protein based on the k-means clustering algorithm. There are 21 nodes, with 32 edges; the average node degree is 3.05 with an average local clustering coefficient of 0.756. Table 4 showcases the detailed results of the interaction analysis.

Amino peptides have been reported to play a crucial role in the formation and metabolism of neuropeptides essential for neurological health. Various studies suggest that amino peptides are sensitive to puromycin and hold myriad central nervous system (CNS) functions such as memory, amnesia, apoptosis, and schizophrenia. On the other hand, ST3GAL4 has been said to promote Parkinson’s disease (PD) because of its decreased expression. The density-regulated protein (DENR) has been shown to promote various diseases in humans namely cancer, neurological disorders, and viral infections. Moreover, overexpression of NPEPPS is responsible for Alzheimer’s and other neurodegenerative dementias collectively called tauopathies. Type-1 angiotensin II receptor-associated proteins have been studied widely for elucidating medical conditions such as blood pressure, electrolyte balance, and renal and neurological, as well as endocrine functions that are associated with cardiovascular control. Another 2014 study reports that Inositol trisphosphate 3-kinase B is increased in the human cerebral cortex of Alzheimer’s patients. Studies have shown that SOCS2 plays an essential role in the central nervous system (CNS), metabolism, immune response, mammary gland development, oncology, and cytokine-dependent signaling pathways. Renin or brain rennin-angiotensin system (RAS) has been proven to have implications in a much broader range of neural effects. Also, the brain RAS is composed of the ACE2, Ang17, and prorenin and Mas receptors responsible for many neural effects.
Table 3. Best 15 characteristic contigs present in the ACE2 gene (Gene ID: 59272).

| CDS Feature | Start | Stop | Function         | Length | Region  |
|-------------|-------|------|------------------|--------|---------|
| Feature 1   | 629   | 835  | Hypothetical protein | 207    |         |
| Feature 2   | 983   | 864  | Hypothetical protein | 120    |         |
| Feature 3   | 1339  | 1211 | Hypothetical protein | 129    |         |
| Feature 4   | 1674  | 1525 | Hypothetical protein | 150    |         |
| Feature 5   | 1710  | 1910 | Hypothetical protein | 201    |         |
| Feature 6   | 2614  | 2486 | Hypothetical protein | 129    |         |
| Feature 7   | 2621  | 2926 | Hypothetical protein | 306    |         |
| Feature 8   | 3134  | 2994 | Hypothetical protein | 141    |         |
| Feature 9   | 3135  | 3284 | Hypothetical protein | 150    |         |
is not a new concept that the growth hormone (GH) and gonadotropin-releasing hormone (GnRH) in the brain are implicated in disorders in the cerebral cortex, hypothalamus, hippocampus, cerebellum, spinal cord, and neural retina, and are also responsible for brain tumors. Both GH and GnRH have shown the potential to cause neurotrophic, neuroprotective, and neuro-regenerative action in the human brain. On the other hand, solute carrier transporters (SLC) play a pivotal role in promoting neuro-degenerative disorders such as Alzheimer’s disease, amyotrophic lateral sclerosis, Huntington’s disease, Parkinson’s disease, depression, post-traumatic stress disorder, dementia, schizophrenia, and epilepsy. Our interaction analysis discerns the evidence: ACE2 and its 10 most significant interactors are related and play a role in promoting neuronal disorders in humans, while ACE2 is also the main entry receptor of SARS-CoV-2. Therefore, it is important to refer here that ACE2 is directly responsible for neurological disorders such as Alzheimer’s disease, Parkinson’s disease, and epilepsy that have been lately observed to rise in COVID-19 patients.

**Gene-Drug Mapping**

Gene drug mapping is an essential step toward understanding how drug compounds function when used to treat various diseases. Every drug candidate has its own potential to bind to a gene/protein target. We deployed the three best gene-drug mapping resources – DGIdb, DrugBank, and PubChem to identify the best drug candidates that have been validated to bind to the seed genes. Most of the synthetic drugs that were identified were mainly inhibiting the seed genes or were antagonists. Table 5 summarises the gene-drug mapping.

**Identification of Alternative Herbal Compounds**

The search for herbal medicines has become necessary to prevent adverse drug reactions (ADRs) and various other side effects that are encapsulated by synthetic medications. Alternative medicine is an umbrella term for a number of therapies that involve the use of dietary supplements, hormones,

| CDS Feature | Start | Stop | Function         | Length |
|-------------|-------|------|------------------|--------|
| Feature 10  | 3346  | 3459 | Hypothetical protein | 114    |
| Feature 11  | 3771  | 3652 | Hypothetical protein | 120    |
| Feature 12  | 3915  | 3778 | Hypothetical protein | 138    |
| Feature 13  | 4018  | 4203 | Hypothetical protein | 186    |
| Feature 14  | 5279  | 5139 | Hypothetical protein | 141    |
| Feature 15  | 5320  | 5547 | Hypothetical protein | 228    |
vitas, proteins, and herbs. Alternative herbal medications are gaining ground due to serious risks posed by highly effective synthetic drugs. We have identified the alternative medications to synthetic drugs via extensive literature mining studies. The identification of alternative herbal compounds as a potential treatment for the neurological manifestations of COVID-19 can be a safe and effective therapy for the said disease manifestations. The herbal source, along with its bioactive phytochemical components, are mentioned in Table 6.

Alkaloids, flavonoids, saponins, terpenoids, and tannins are major phytochemical classes that can be used to treat the neurological dysfunctions that are associated with COVID-19 in patients. Phytochemicals have already been established to provide protection against numerous diseases such as cancer, diabetes, cardiac problems, and even neurological disorders. Alkaloids are known to provide protection against neurological diseases such as febrile epilepsies, cerebral ischemia, oblivion, major depressive disorders (MDD), anxiety, and autism. Flavonoids on the other hand associate with different pathways such as ERK and PI3-kinase/Akt and regulate the dynamics, in turn producing neuroprotective effects. They have a tendency to disturb and inhibit neuronal apoptosis that is triggered by neurotoxic substances in neurodegenerative diseases. Because of the inhibitory actions of flavonoids, these are prescribed mainly to treat dementia or oblivion or age-related neurological disorders (Alzheimer’s disease) in people.

Tannins, also known as polyphenols, encapsulate myriad health benefits. They are omnipresent in vegetables and fruits. They are known to possess anti-oxidant, anti-aging, anti-inflammatory, and anti-cancer properties, but can also be deployed to treat neurological problems such as Alzheimer’s disease (AD) and Parkinson’s disease (PD). Similarly, another important group of bioactive phytochemicals, saponins, are simply either glycosides of triterpenoid or steroidal aglycones. They have been widely deployed in traditional Chinese medicines (TCMs). Evidence suggests that saponins are beneficial to treat neurodegenerative and psychological diseases such as stroke, Alzheimer’s disease, Parkinson’s disease, and Huntington’s disease. Terpenoids have been suggested to be anti-convulsants that can be deployed to treat schizophrenia, insomnia, anxiety, pain, and cognitive problems.
Pharmacological Screening of Phytochemicals

The identified phytochemicals were then subjected to ADMET screening. For the pharmacological assessment, the main criteria for the selection of the phytochemicals were i) Druglikeness, ie, based on the Lipinsky’s Rule of 5 (RO5), where the compounds must have a) \( \log P < 5 \) (milog), b) molecular weight \(< 500 \) (MW), c) number of hydrogen bond acceptors \(< 10 \) (nO), d) number of hydrogen bond donors \(< 5 \) (nOHNH) and e) the number of molecules violating more than 1 of these rules should be 0; ii) Medicinal chemistry that refers to the lead-likeness of a compound, bioavailability score and synthetic accessibility. Out of 35 identified phytochemicals, only 6 passed the ADMET evaluation. These were andrographolide, 14-deoxy-11,12-didehydroandrographolide, terphenolide, safranal, zingerone and p-cineole. Table 7 depicts the pharmacological screening results for the six qualified phytochemicals.

Table 4. Significant 10 interactors found in ACE2 physical network.

| S.No. | Interacting Partner | Description | Confidence Score |
|-------|---------------------|-------------|------------------|
| 1.    | Aminopeptidase Q (AQPEP) | A metalloprotease is important for placentation by regulating biological activity of key peptides at the embryo- maternal interface. | 0.621 |
| 2.    | CMP-N-acetylneuraminate-β-galactosamide-α-2,3-sialytransferase 4 (ST3GAL4) | It catalyses the formation of the NeuAc-α-2,3-Gal-β-1,4-GlcNAc-, and NeuAc-α-2,3-Gal-β-1,3-GlcNAc- sequences found in terminal carbohydrate groups of glycoproteins and glycolipids. | 0.619 |
| 3.    | Density-regulated protein (DENR) | Important for translation of target mRNAs by scanning and recognition of the initiation codon. Also, it plays a role in translation initiation; promotes the recruitment of aminoacylated initiator tRNA to P site of 40S ribosomes. Can promote release of decacylated tRNA and mRNA from recycled 40S subunits following ABCE1-mediated dissociation of post-termination ribosomal complexes into subunits. | 0.542 |
| 4.    | Puromycin-sensitive aminopeptidase (NPEPPS) | Plays a role in proteolytic events important for cell growth and viability. Also, it acts as a regulator of neuropeptide activity. | 0.539 |
| 5.    | Type-1 angiotensin II receptor-associated protein (AGTRAP) | A negative regulator of type-1 angiotensin II receptor-mediated signaling by regulating receptor internalization, as well as the mechanism of receptor desensitization such as phosphorylation. Also decreases in cell proliferation and angiotensin II-stimulated transcriptional activity. | 0.517 |
| 6.    | Inositol-trisphosphate 3-kinase B (ITPKB) | - | 0.452 |
| 7.    | Suppressor of cytokine signaling 2 (SOCS2) | Form part of a classical negative feedback system that regulates cytokine signal transduction. | 0.452 |
| 8.    | Renin (REN) | An endopeptidase whose known function is to generate angiotensin I from angiotensinogen in the plasma, initiating a cascade of reactions that produce an elevation of blood pressure and increased sodium retention by the kidney. | 0.444 |
| 9.    | Growth hormone-releasing hormone receptor (GHRHR) | A receptor for GRF, coupled to G proteins that promote adenylyl cyclase. It stimulates somatotroph cell growth, growth hormone gene transcription, and growth hormone secretion | 0.428 |
| 10.   | Solute carrier family 15 member 1 (SLC15A1) | Solute carriers. | 0.426 |

Binding Cavity Prediction and Molecular Docking

There were mainly six pockets that were identified in receptor ACE2. The highest scoring pocket was pocket 1, which had 139 surface atoms. This pocket had a good probability of fitting a ligand \( (P = 0.93) \). It spanned a larger surface area of
the ACE2 protein and suggested a central ligand docking. That simply indicates that any ligand/molecule irrespective of its size can easily fit inside this cavity. Furthermore, pocket 1 includes 139 residues. Out of these, position 273 is known to be a binding site for substrate \(^{58}\); position 374 is a metal binding site \(^{58}\); position 375 is discerned to be an active site \(^{58, 59}\); and position 515 is the substrate binding site region \(^{58}\). The rest of the binding cavities identified are mostly present on the surface. Table 8 displays the details of each binding cavity.

Supplementary file S2 shows a video that displays the two main pockets – 1 (in blue) and pocket 2 (in red) (Fig. 2).

After docking, we observed that only andrographolide (score = −7.8) and 14-deoxy-11,12-didehydroandrographolide (score = −8.0) had a greater binding affinity towards receptor ACE2 (Table 9). Additionally, it is noteworthy that both these phytochemicals are from *Andrographis paniculata*, and occupied a cavity space of 926, indicating their bigger ligand size. The bigger the ligand size, the greater the protein structural conformations are observed.

**Electrostatic Computation**

After molecular docking, it was evident that only andrographolide and 14-deoxy-11,12-didehydroandrographolide have a greater binding affinity toward the ACE2 receptor. Therefore, only these two complexes were further selected for an electrostatic analysis to check the overall energy of the systems along with the macromolecular energy landscape. Energy assessment plays an important role after molecular docking and simulation. It is so because when a ligand is docked to a receptor, especially a protein structure, it tends to alter the conformation of the protein. This alteration in the protein pocket eventually dictates the effectiveness or ineffectiveness of a ligand. If the docked ligand comfortably fits inside the pocket of the protein receptor, it indicates that the complex will not have enormous unstable energy. It will also tend to have little to no steric hindrance. Table 10 summarizes the electrostatics computed for the two selected complexes.

![Image](https://via.placeholder.com/150)
Table 6. Alternative herbal compounds.

| Synthetic drug               | Herbal Source                                                                 | Phytochemical present                                                                 | Reference |
|------------------------------|-------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|-----------|
| Bromhexine                   | Andrographis paniculata and ivy/primrose/thyme-based preparations and moderate evidence for Pelargonium sidoides | Andrographolide, 14-deoxy-11,12-didehydroandrographolide and 14-deoxy andrographolide | 47–49     |
| Chloroquine                  | Leaf preparations of Kalanchoe pinnata                                         | Flavonoids and Methanol extract.                                                       | 50        |
| N-(2-Aminoethyl)-1-aziridineethanamine Tosedostat | Hibiscus cannabinus (Kena), Euphorbia formosana, Allium sativum, Moringa oleifera, Vernonia amygdalina, Achillea fragrantissima, Typhonium flagelliforme | Alicin, S-allyl cysteine (SAC), Diallyl disulfides (DADS), Diallyl trisulfides (DATS), and Methyl thiosulfonate | 49–53     |
| Carbocisteine                | Licorice, Eucalyptus, Hedera helix leaves                                      | Glycyrrhizic acid (Triterpenoid saponin)                                               | 49, 54    |
| Potassium nitrate            | Osmium basilicum                                                              | Alkaloids, Terpenoids, Ascorbic acid, Flavanoids, Soybean glycosides, Tannins.        | 49, 50, 53–57 |
| Artenimol                    | Bucoas semperfivus, Hibiscus sabdariffa                                       | N/A                                      | 54        |
| Valsartan                    | Achillea wilhelmsii, Hibiscus sabdariffa, Ganoderma lucidum, Allium sativum   | Anthocyanins                            | 50        |
| Olmesartan                   | Hibiscus sabdariffa                                                           | Anthocyanins                            | 50        |
| Losartan                     | Camellia sinensis, Crocus sativus                                             | Safranaland Crocetin                    | 47, 49    |
| Candesartan                  | Coptis chinensis                                                              | Berberine (BBR)                         | 47        |
| Cilexetril                   | Coptis chinensis                                                              | Berberine (BBR)                         | 47        |
| Eprosartan                   | Coptis chinensis                                                              | Berberine (BBR)                         | 47        |
| Telmisartan                  | Coptis chinensis                                                              | Berberine (BBR)                         | 47        |
| Irbesartan                   | Coptis chinensis                                                              | Berberine (BBR)                         | 47        |
| Forsartan                    | Coptis chinensis                                                              | Berberine (BBR)                         | 47        |
| Saprisartan                  | Coptis chinensis                                                              | Berberine (BBR)                         | 47        |
| Tasosartan                   | Coptis chinensis                                                              | Berberine (BBR)                         | 47        |
| Cetrimonide                  | Allium cepa, Allium angolense                                                 | Flavonoids                              | 50        |
| Bicalutamide                 | Coriama aromatica                                                            | Cucumin                                  | 50, 55–57 |
| Docetaxel                    | Zingiber officinale                                                          | Zingerone                                | 57        |
| Aliskiren                    | Zingiber officinale                                                          | Camphene, α-terpinene, farnesene, p-cineole, zingiberene, β-myrcene                    | 55, 57    |
| Tesamorelin                  | N/A                                                                           | N/A                                      | -         |
| Ramipril                     | Hibiscus                                                                     | Anthocyanins                            | 50        |
| Oselamvivir                  | Nigella sativa                                                               | Thymoquinone, carvacrol, longifolene, 4-terpineol, r-anetohle, limonene, thymol         | 49, 57    |
| Midoctine                    | Carynanthoe yolbiniae                                                        | Indole alkaloids                         | 50        |
| Cefmetazole                  | Lepidium sativum (≡ Cardamom sativum)                                        | Thujene, myrcene                         | 55, 57    |
| Ampicillin                   | Cinnamomum zeillicium, Daucus carota, Eucalyptus globulus, Rasmarinus officinalis | 1,8-Cineole (ethyl-dimethyl-(3-sulfopropyl) azanium) (C₁₈H₁₄NO₅S⁺) | 55, 57    |

Table 7. Best phytochemicals obtained after the pharmacological screening.

| Phytochemical                      | GI absorption | BBB permeant | Pgp substrate | Bioavailability Score | Synthetic Accessibility | PAINS |
|------------------------------------|---------------|--------------|---------------|------------------------|--------------------------|-------|
| Andrographolide                    | High          | No           | Yes           | 0.55                   | 5.06                     | 0     |
| 14-deoxy-11,12-didehydroandrographolide | High         | Yes          | No            | 0.55                   | 5.29                     | 0     |
| Terphenolide                       | High          | Yes          | No            | 0.56                   | 4.02                     | 0     |
| Safranal                           | High          | Yes          | Yes           | 0.55                   | 3.14                     | 0     |
| Zingerone                          | High          | No           | No            | 0.56                   | 3.42                     | 0     |
| p-cineole                          | High          | Yes          | Yes           | 0.55                   | 2.23                     | 1     |
energy (−7448.2 kJ/mol). The minimal residual fluctuations have been represented in green links, while maximum residual fluctuations have been depicted in red. The MM-GBSA has been represented in the form of an APBS map. The APBS range for the complex was recorded to lie between −605.7 to 475.9. This score further confirms the stability of the phytochemical inside the main pocket (pocket 1) of the ACE2 receptor.

Discussion

Since the COVID-19 pandemic broke out in early 2020, life has been turned upside-down for one and all. Because of drastic life changes, people have not only been infected by the SARS-CoV-2 virus, but have also experienced neurological and psychological problems, such as depression, anxiety, insomnia, brain fog, and epilepsy. Angiotensin-converting enzyme 2 (ACE2) has been discerned to be the entry receptor of SARS-CoV-2. Therefore, we tried to understand how the ACE2 receptor is involved in the COVID-19 disease by exploring genomic and epinformatics domains. By motif enrichment, we identified only two motifs, motif M2 and M5. Motif M2 (AGTACTGCC, score = 10.6676) is present on the negative strand and has a length of 9 nucleotides, while motif M5 (AGTACTGTAGATGGTGCTCA, score = 11.8863) is also located on the negative strand and has a length of 20 nucleotides. These two motifs are said to be crucial, as they were a perfect match for zinc fingers, DNA-binding domains, heart and neural crest derivatives, tRNA-methyltransferases, and paired like homeoboxes present in the human, mouse, and yeast genome. Furthermore, by analyzing the genomic annotation, we found that the GC content directly affects the genome functioning and species ecology of the ACE2 gene, and it was observed to be 38.5% showing the quadratic relationship with the genomic size of the ACE2 gene. The analogy is simple - the larger the genomic size, the lower the GC content viz., because of the higher biochemical expenditure of GC base formation.

Table 8. Predicted binding cavities present in ACE2 receptor.

| Pocket name | Rank | Score | Probability | Sas_points | Surf_atoms | Center_X | Center_Y | Center_Z |
|-------------|------|-------|-------------|------------|------------|----------|----------|----------|
| Pocket 1    | 1    | 32.58 | 0.93        | 283        | 139        | −78.9507 | 8.6355   | −0.7977  |
| Pocket 2    | 2    | 2.40  | 0.064       | 8          | 9          | −84.4854 | 18.733   | 13.6999  |
| Pocket 3    | 3    | 2.12  | 0.048       | 12         | 11         | −81.6689 | −7.4151  | −8.0919  |
| Pocket 4    | 4    | 1.56  | 0.023       | 12         | 13         | −87.755  | 10.4459  | 13.8753  |
| Pocket 5    | 5    | 1.31  | 0.015       | 22         | 21         | −72.5893 | −12.0819 | −5.5877  |
| Pocket 6    | 6    | 1.01  | 0.007       | 21         | 11         | −74.8345 | 15.2118  | −9.6363  |

Fig 2. Six best phytochemicals docked to receptor ACE2.
We identified clusters of orthologs (COGs) for the ACE2 protein sequence. The identified COGs play crucial roles in biological processes such as cell cycle regulation; translation; RNA processing and modification; chromatin structure and dynamics; post-translational modifications (PTMs); intracellular trafficking, secretions, and vesicular control. However, only COG Oligoendopeptidase F (COG1164) was found to be more significant for the ACE2 protein receptor. The ACE2 protein was further subjected to network analysis to identify its associated interacting partners. There were 10 significant interacting partners of our query protein ACE2, namely AQPEP, ST3GAL4, DENR, NPEPPS, AGTRAP, ITPKB, SOCS2, REN, GHRHR, and SLC15A1. These network associators provide insights into the possible neurological dysfunctions that could be occurring in the patients who were infected with COVID-19. Therefore, we executed a gene-drug mapping to screen for highly potent synthetic drug candidates for each of these seed genes.
identified, their alternative herbal phytochemicals were screened by literature mining. It was found that alkaloids, flavonoids, saponins, terpenoids, and tannins can be used to treat the neurological dysfunctions that are associated with COVID-19 in patients. On subjecting the identified phytochemicals to pharmacological, pharmacokinetic (PK), and pharmacodynamic (PD) analysis, only 6 out of a total of 35 passed the ADMET evaluation. These were andrographolide, 14-deoxy-11,12-didehydroandrographolide, terphenolide, safranal, zingerone and p-cineole. These six phytochemicals were then submitted for molecular docking using PyRx software. The highest binding affinity with the ACE2 receptor was noted for andrographolide and 14-deoxy-11,12-didehydroandrographolide. Although, both displayed a good binding affinity, 14-deoxy-11,12-didehydroandrographolide bound to the ACE2 receptor with much more stable energy (~158041.728091 kJ/mol), with minimal energy fluctuations when compared to andrographolide with ACE2. Therefore, we discern andrographolide and 14-deoxy-11,12-didehydroandrographolide, both major constituents of Andrographis paniculata, must be further clinically validated for treating neurological dysfunctions associated with the ACE2 receptor in COVID-19 patients.

Herbal compounds are known to be important sources for developing therapeutics for diseases. Often with fewer to no adverse reactions and low molecular weight, they often showcase a good binding towards protein receptors. In order to prevent a disease from occurring or reducing its effect, it becomes important to block the enzyme’s catalytic activity. Thus, herbal compounds such as the diterpenoids andrographolide and 14-deoxy-11,12-didehydroandrographolide can be used as natural inhibitors.

A. paniculata is known for its anti-inflammation, anti-bacterial, anti-oxidant, and anti-cancer properties, and forms one of the important Ayurvedic formulas in Indian Ayurvedic medicine. The plant is native to South Asia and is found in India and Sri Lanka. The constituents of the plant are well-recognized for viral and bacterial infections. However, there is also a need to pay heed to their efficacy towards neurological dysfunctions.

Conclusion

Angiotensin-converting enzyme 2 (ACE2) is known to be the entry receptor of the SARS-CoV-2 virus, and is responsible for various neurological dysfunctions that are associated with COVID-19 in patients. To treat the neurological manifestations associated with the ACE2 receptor, instead of synthetic medications, we discern that the phytochemicals andrographolide and 14-deoxy-11,12-didehydroandrographolide, both major constituents of the plant Andrographis paniculata, must be further clinically validated for treating neurological dysfunctions associated with the ACE2 receptor in COVID-19 patients.

Acknowledgements

SQ is supported by a DST-INSPIRE fellowship provided by the Department of Science & Technology (DST), Govt. of India.

Conflict of Interest

None

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Author JH acknowledges funding from “Study on the effect of low oxygen inhalation in patients humidification”, and “Study on nursing effect of psychological intervention guidance in emergency patients with multiple injuries”.

ORCID iDs

Shaban Ahmad https://orcid.org/0000-0001-9832-2830
Khalid Raza https://orcid.org/0000-0002-3646-6828

Supplemental Material

Supplemental material for this article is available online.

References

1. Jit BP, Qazi S, Arya R, et al. An immune epigenetic insight to COVID-19 infection. Epigenomics. 2021;13(6):465-480. doi:10.2217/epi-2020-0349.
2. Qazi S, Sheikh K, Faheem M, et al. A coadunation of biological and mathematical perspectives on the pandemic COVID-19: a review. Corronaviruses. 2021;2(9):e030821190295. doi: 10.2174/266679670266621014110013.
3. Qazi S, Sheikh K, Raza K. In silico approach to understand the epigenetic mechanism of SARS-CoV-2 and its impact on the environment. VirusDisease. 2021;32(2):286-297. doi:10.1007/s13337-021-00655-w.
4. Sultana S, Ananthapur V. COVID-19 and its impact on neurological manifestations and mental health: the present scenario. Nutrol. Sci. 2020;41(11):3015-3020. doi:10.1007/s10072-020-04695-w.
5. CDC. About variants of the virus that causes COVID-19. 2021. https://www.cdc.gov/coronavirus/2019-ncov/transmission/variant.html (Accessed on 19th May, 2021).
6. Muralidharan N, Sakthivel R, Velmurugan D, et al. Computational studies of drug repurposing and synergism of lopinavir, oseltamivir and ritonavir binding with SARS-CoV-2 protease against COVID-19. J. Biomol. Struct. Dyn. 2020;39(7):2673-2678. doi: 10.1080/07391102.2020.1752802.
7. Dong L, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19). Drug Discov Ther. 2020;14(1):58-60. doi:10.5582/dht.2020.01012.
8. Farooq S, Ngaini Z. Natural and synthetic drugs as potential treatment for coronavirus disease 2019 (COVID-19). Chemistry Africa. 2021;4(1):1-13. doi: 10.1007/s42250-020-00203-x.
9. Isa MA, Mustapha A, Qazi S, et al. In silico molecular docking and molecular dynamic simulation of potential inhibitors of 3C-like main protease (3CLpro) from severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) using selected African medicinal plants. Adv. Trad. med. 2022;22:107-123. doi: 10.1007/s13596-020-00523-w.
10. Khuntia BK, Sharma V, Qazi S, et al. Ayurvedic medicinal plants against COVID-19: an in silico analysis. *Nat. Prod. Commun.* 2021;16(11):1934578x2110567. doi: 10.1177/1934578x21105675.

11. Acharya A, Kevadiya BD, Gendelman HE, Byrareddy SN. SARS-CoV-2 infection leads to neurological dysfunction. *J Neuroimmune Pharmacol.* 2020;15(2):167-173. doi: 10.1007/s11481-020-09924-9.

12. Panariello F, Cellini I, Speciani M, et al. How does SARS-CoV-2 affect the central nervous system? A working hypothesis. *Front. Psychiatry.* 2020;11:582345. doi: 10.3389/fpsyg.2020.582345.

13. Xu J, Lazartigues E. Expression of ACE2 in human neurons supports the neuro-invasive potential of COVID-19 virus. *Cell. Mol. Neurobiol.* 2020;42(1):305-309. doi: 10.1007/s10571-020-00915-1.

14. Kase Y, Okano H. Neurological pathogenesis of SARS-CoV-2 (COVID-19): from virological features to clinical symptoms. *Neurobiol. Psychiatry.* 2020;42(1):305-309. doi: 10.1007/s10571-020-00915-1.

15. Ziebarth JD, Bhattacharya A, Cui Y. CTCFBSDB 2.0: a database for CTCF-binding sites and genome organization. *Nucleic Acids Res.* 2013;41(D1):D188-D194. doi: 10.1093/nar/gks1165.

16. Bao L, Zhou M, Cui Y. CTCFBSDB: a CTCF binding site database for characterization of vertebrate genomic insulators. *Nucleic Acids Res.* 2008;36:D83-D87. doi: 10.1093/nar/gkm875.

17. Bailey TL, Machanick P. Inferring direct DNA binding from ChIP-seq. *Nucleic Acids Res.* 2012;40:e128. doi: 10.1093/nar/gks433.

18. Gupta S, Stamatoyannopoulos JA, Bailey TL, Noble WS. Quantifying similarity between motifs. *Genome Biol.* 2007;8(2):R24. doi: 10.1186/gb-2007-8-2-r24.

19. Aziz RK, Bartels D, Best A, et al. The RAST server: rapid annotations using subsystems technology. *BMCMicrobiol.* 2008;8:97. doi: 10.1186/1471-2180-8-97.

20. Wu S, Zhu Z, Fu I, Niu B, Li W. WebMGA: a customizable web server for fast metagenomic sequence analysis. *BMCMicrobiol.* 2011;11:12444. doi: 10.1186/1471-2164-12-444.

21. Szklarczyk D, Gable AL, Lyon D, et al. STRING V11: protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* 2019;47(D1):D607-D613. doi: 10.1093/nar/gky1131.

22. Freshour SL, Kiwala S, Cotto KC, et al. Integration of the Drug–Gene Interaction Database (DGIdb 4.0) with open crowdsource efforts. *Nucleic Acids Res.* 2020;49(D1):D1144-D1151. doi: 10.1093/nar/gkaa1084.

23. Wishart DS. Drugbank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res.* 2006;34(90001):D668-D672. doi: 10.1093/nar/gkj067.

24. Kim S, Thiesen PA, Bolton EE, et al. Pubchem substance and compound databases. *Nucleic Acids Res.* 2015;44(D1):D1202-D1213. doi: 10.1093/nar/gkv951.

25. Gaulton A, Bellis LJ, Bento AP, et al. ChEMBL: a large-scale bioactivity database for drug discovery. *Nucleic Acids Res.* 2011;40(D1):D1100-D1107. doi: 10.1093/nar/gkr777.

26. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and chemical property friendliness of small molecules. *Sci. Rep.* 2017;7:42717. doi: 10.1038/srep42717.

27. Jendele I, Krivak R, Skoda P, et al. Prankweb: a web server for ligand binding site prediction and visualization. *Nucleic Acids Res.* 2019;47(W1):W334-W349. doi: 10.1093/nar/gka424.

28. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. *Methods Mol. Biol.* 2015;1263:243-250. doi: 10.1007/978-1-4939-2269-7_19.

29. Walsh I, Minervini G, Corazza A, et al. Bluese server: electrostatic properties of wild-type and mutated protein structures. *Bioinformatics.* 2012;28(16):2189-2190. doi: 10.1093/bioinformatics/bts343.

30. Parra RG, Schafer NP, Radusky LG, et al. Protein Frustratometer 2: a tool to localize energetic frustration in protein molecules, now with electrostatics. *Nucleic Acids Res.* 2016;44(W1):W356-W360. doi: 10.1093/nar/gkw304.

31. Fornes O, Castro-Mondragon JA, Khan A, et al. JASPAR 2020: update of the open-access database of transcription factor binding profiles. *Nucleic Acids Res.* 2020;48(D1):D87-D92. doi: 10.1093/nar/gka1001.

32. Newburger DE, Bulyk ML. UniPROBE: an online database of protein binding microarray data on protein-DNA interactions. *Nucleic Acids Res.* 2009;37(Database):D77-D82. doi: 10.1093/nar/gkn660.

33. Hui KS. Neuropeptidases. *Handbook of Neurochemistry and Molecular Neurobiology: Neuro Protein Metabolism and Function.* 2007;625-651. doi: 10.1007/978-0-387-30379-6_21.

34. Herman ZS, Stachura Z, Laskawie G, et al. Antinociceptive effects of puromycin and bacitracin. *Pol. J. Pharmacol.* 1985;37(2):133-140.

35. Eisenstein EM, Altman HJ, Barraco DA, et al. Brain protein synthesis and memory: the use of antibiotic probes. *Fed. Proc.* 1983;42(14):3080-3085.

36. Tobler AR, Constan DB, Schmitt-Gräff A, et al. Cloning of the human puromycin-sensitive aminopeptidase and evidence for expression in neurons. *J. Neurochem.* 1997;68(3):889-897. doi: 10.1046/j.1471-4159.1997.68030889.x.

37. Hui M, Badai ED, Lajtha A, et al. Changes in puromycin-sensitive aminopeptidases in postmortem schizophrenic brain regions. *Neurochem. Int.* 1995;27(4-5):433-441. doi: 10.1016/0197-0186(95)00025-4.

38. Schneider JS. Altered expression of genes involved in ganglioside biosynthesis in substantia nigra neurons in Parkinson’s disease. *PloS one.* 2018;13(6):e0199189. doi: 10.1371/journal.pone.0199189.

39. Lomakin IB, Dmitriev SE, Steitz TA. Crystal structure of the DENR-MCT-I complex revealed zinc-binding site essential for heterodimer formation. *PNAS.* 2019;116(2):528-533. doi: 10.1073/pnas.1809681116.

40. Kudo LC, Parfenova L, Ren G, et al. Puromycin-sensitive amino- peptidase (PSA/NPEPPS) impedes development of neuropathology in hPSA/TAU(P301L) double-transgenic mice. *Polymer.* 2011;20(9):1820-1833. doi: 10.1093/hmg/ddr065.

41. Guo DF, Sun YL, Hamet P, Inagami T. The angiotensin II type 1 receptor and receptor-associated proteins. *Cell Res.* 2001;11(3):165-180. doi: 10.1038/sj.cr.7290083.

42. Stygelbout V, Leroy K, Pouillon V, et al. Inositol trisphosphate 3-kinase B is increased in human Alzheimer brain and exacerbates mouse Alzheimer pathology. *J. Neuro.* 2014;137(Pt 2):537-552. doi: 10.1093/brain/awt344.
43. Rico-Bautista E, Flores-Morales A, Fernández-Pérez L. Suppressor of cytokine signaling (SOCS) 2, a protein with multiple functions. Cytokine Growth Factor Rev. 2006;17(6):431-439. doi: 10.1016/j.cytogfr.2006.09.008.

44. Phillips MI, de Oliveira EM. Brain renin angiotensin in disease. J. Mol. Med. 2008;86(6):715-722. doi: 10.1007/s00109-008-0331-5.

45. Martínez-Moreno CG, Calderón-Vallejo D, Harvey S, et al. Growth hormone (GH) and gonadotropin-releasing hormone (GnRH) in the central nervous system: a potential neurological combinatorial therapy? Int. J. Mol. Sci. 2018;19(2):375. doi: 10.3390/ijms19020375.

46. Ayka A, Şehirli AO. The role of the SLC transporters protein in the neurodegenerative disorders. Clin Psychopharmacol Neurosci. 2020;18(2):174-187. doi:10.9758/cpn.2020.18.2.174.

47. Affuso F, Mercurio V, Fazio V, et al. Cardiovascular and metabolic effects of Berberine. World J. Cardiol. 2010;2(4):71-77. doi:10.4330/wjc.v2.i4.71.

48. Wagner L, Cramer H, Klose P, et al. Herbal medicine for cough: a systematic review and meta-analysis. Forsch Komplementmed. 2015;22:359-368. doi: 10.1159/000442111.

49. Matayi A, Nabhvi SM, Daglia M, Jafari S. Natural terpenoids as a promising source for modulation of GABAAergic system and treatment of neurological diseases. Pharma Rep. 2016;68(4):671-679. doi:10.1016/j.pharep.2016.03.014.

50. Ayaz M, Sadiq A, Junaid M, et al. Flavonoids as prospective neuroprotectants and their therapeutic propensity in aging associated neurological disorders. Front Aging Neurosci. 2019;11:155. doi:10.3389/fnagi.2019.00155.

51. Willcox ML, Bodeker G. Traditional herbal medicines for malaria. BMJ (Clinical Research ed.). 2004;329(7475):1156-1159. doi: 10.1136/bmj.329.7475.1156.

52. Saedi TA, Md Noor S, Ismail P, Othman F. The effects of herbs and fruits on leukaemia. Evid Based Complement Alternat Med. 2014;2014:494136. doi:10.1155/2014/494136.

53. Al Dsi SS, Anwar MA, Eid AH. Anti-hypertensive herbs and their mechanisms of action: part I. Front Pharmacol. 2016;6:323. doi: 10.3389/fphar.2015.00323.

54. Sun A, Xu X, Lin J, et al. Neuroprotection by saponins. Phytother Res. 2014;29(2):187-200. doi: 10.1002/ptr.5246.

55. Hussain G, Rasul A, Anwar H, et al. Role of plant derived alkaloids and their mechanism in neurodegenerative disorders. Int J Biol Sci. 2018;14(3):341-357. doi:10.7150/ijbs.23247.

56. Singh AP, Kumar S. Applications of tannins in industry. In: Aires A, ed. Tannins - Structural Properties, Biological Properties and Current Knowledge [Internet]. IntechOpen; 2019. doi:10.5772/intechopen.85984.

57. Hussain G, Huang J, Rasul A, et al. Putative roles of plant-derived tannins in neurodegenerative and neuropsychiatry disorders: an updated review. Molecules. 2019;24(12):2213. doi:10.3390/molecules24122213.

58. Towler P, Staker B, Prasad SG, et al. ACE2 x-ray structures reveal a large hinge-bending motion important for inhibitor binding and catalysis. J. Biol. Chem. 2004;279(17):17996-18007. doi: 10.1074/jbc.m311191200.

59. Wang W, McKinnie SMK, Farhan M, et al. Angiotensin-converting enzyme 2 metabolizes and partially inactivates Pyr-apelin-13 and apelin-17. Hypertension. 2016;68(2):365-377. doi: 10.1161/hypertensionaha.115.06892.

60. Abubakar MB, Usman D, El-Saber Batiha G, et al. Natural products modulating angiotensin converting enzyme 2 (ACE2) as potential COVID-19 therapies. Front Pharmacol. 2021;12:629935. doi:10.3389/fphar.2021.629935.

61. Liu YT, Chen HW, Lii CK, et al. A diterpenoid, 14-deoxy-11,12-didehydroandrographolide, in Andrographis paniculata reduces steatohepatitis and liver injury in mice fed a high-fat and high-cholesterol diet. Nutrients. 2020;12(2):523. doi:10.3390/nu12020523.

62. Okturkurobo A, Ehlizogic Fakolun J, Erharuyi O, et al. Harnessing the medicinal properties of Andrographis paniculata for diseases and beyond: a review of its phytochemistry and pharmacology. Asian Pac. J. Trop. Dis. 2014;4(3):213-222. doi: 10.1016/s2222-1808(14)60509-0.

63. Kumar V. Perspective of Andrographis paniculata in neurological disorders. Clin. Pharm Biopharm. 2014;S2(e001):005. doi: 10.4172/2167-065X.s2-005.