Applied Biochemistry and Biotechnology
https://doi.org/10.1007/s12010-022-03827-6

ORIGINAL ARTICLE

In Silico Studies of Phytoconstituents from *Piper longum* and *Ocimum sanctum* as ACE2 and TMRSS2 Inhibitors: Strategies to Combat COVID-19

Divya Jindal[1] · **Vibha Rani**[1]

Received: 25 July 2021 / Accepted: 21 January 2022

Abstract
The recent pandemic due to the COVID-19 virus has caused a global catastrophe. ACE2 and TMPRSS2 are recognized as key targets for viral entry into the host cells. The pandemic has led to the utilization of many synthetic drugs; however, due to various side effects, there is still no effective drug available against the virus. Several natural approaches have been devised, including herbal and ayurvedic medicines, that have proven to be effective against the COVID-19 virus. In the present study, the effect of phytocompounds of *Piper longum* and *Ocimum sanctum* on ACE2 and TRMPSS2 proteins has been studied. The in silico study is done using computational tools of networks of protein–protein interaction, molecular docking, and drug assessment in terms of physicochemical properties, drug-likeness, lipophilicity, water solubility, and pharmacokinetics. Out of selected phytoconstituents, vicenin 2, rosmarinic acid, and orientin were found to have the highest efficacy in terms of molecular interaction and drug-likeness properties against ACE2 and TMPRSS2 host receptor proteins. Our in silico study proposes the therapeutic potential of phytocompounds from *Piper longum* and *Ocimum sanctum* in modulating ACE2 and TMPRSS2 expression. Targeting ACE2 and TMPRSS2 against the SARS-CoV2 by phytomolecules can serve as a rational approach for designing future anti-COVID drugs.

Keywords ACE2 · TMPRSS2 · Phytotherapeutics · COVID-19 · *Piper longum* · *Ocimum sanctum* · In silico

Introduction

The witnessed outbreak of COVID-19 in 2020, caused by coronavirus familyCoronaviridae, specifically SARS-CoV2, leads to a life crisis of 100,000,000 people with more than 2,200,000 deaths worldwide, lasting for around 2 years now. A major concern is their mortality rate which is increasingly ranging from 1 to > 5% [1]. There are various forms of respiratory syndromes such as MERS and SARS, caused by the family of coronavirus [2].

✉ Vibha Rani
vibha.rani@jiit.ac.in

[1] Department of Biotechnology, Jaypee Institute of Information Technology (JIIT), Noida, U.P, India

Among all, recently identified COVID-19 has been declared as "novel coronaviruses" by WHO [3]. The virus SARS-CoV has shown a 79.5% sequence similarity of the genome with members of the coronavirus family [4].

The two major proteins involved in the entry of the virus are ACE (angiotensin-converting enzyme) and TMPRSS2. ACE is found to be located at chromosome no. 7, encoding to 180 KDa protein involving two homologous domains [5]. ACE is a transmembrane protein, anchored with the carboxy-terminal domain, functioning in hydrolyzing circulating peptides. In 2000, ACE2 has been cloned, being the first human ACE homolog, and mapped onto X-chromosome, forming an 805 amino acid having an extracellular catalytic domain weighing about 120 KDa [6]. The protein ACE2 is surrounded by loop ridges, helices, and some parts of beta-sheets. ACE2 constitutes of two domains, a carboxy-terminal domain, which helps in receptor binding and another is an amino-terminal domain, comprising of one zinc metallopeptidase active site [7]. ACE2, a homolog of ACE, acts on two different peptides substrates, cleaving AngI and AngII [8]. Angiotensin, on the action of Renin, is converted into Angiotensin I and generating Angiotensin 1–9 on the action of ACE2, whereas Angiotensin I is altered into Angiotensin II and generating Angiotensin 1–7 by ACE2. This Angiotensin II is involved in vasoconstriction, hypertension, and cardiac hypertrophy; however, Angiotensin 1–7 is involved in vasodilatation and hypotension [9].

SARS-CoV uses functional ACE2 receptors for their entry inside the host cell [10, 11]. ACE2 binds to SARS-CoV spike protein and is supported by "syncytia formation," which is made up of a fusion of spike protein cells into multinucleated cells [12] and replicates via some cyclic processes [13]. The entry of the virus is mediated by the binding of the N-terminal S1 unit of viral protein to the ACE2 receptor, followed by cleavage amidst S1 and S2 units, which are activated by TMPRSS2 [14]. When the S1 unit is detached, the S2 unit undergoes a conformational change and fuses with the cell membrane, and releases its DNA, further initiating their replication and infection into the host cell. Generally, ACE2 receptors are expressed in the heart, vessels, gut, lung, kidney, testis, and brain [15–18]. In the current pandemic situation, ACE2 receptors are contemplated as "devils," ensuing the entry for the virus, and on the other side as it provides a protective function of degrading Angiotensin II to Angiotensin 1–7, reducing their adverse effects on cells [19]. Targeting ACE2 may be a rational approach for designing anti-COVID drugs.

Another important protein is TMPRSS2, a transmembrane protease serine 2, located at chromosome no. 21 which is crucial for viral infection by assisting the virus to fuse across the cell membrane through ACE2. TMPRSS2 gene contains many androgen receptor elements (AREs), positioned upstream to the start site [20]. It encodes for 92 amino acids which are transmembrane-associated and converts through autocatalytic cleavage at Arg 225 and Ile 256. The cleaved unit is released in the extracellular matrix. The entry and fusion of enveloped viruses are controlled by glycoproteins [21, 22]. Being a class I viral fusion protein, spike protein contains two domains, S1 and S2 domains. S1 is involved in receptor binding, and S2 assists infusion.

The SARS-CoV enters cells via two pathways, one is through TMPRSS2, and the other is mediated by cathepsin LB in the endosome [23, 24]. This protein cleaves S glycoprotein and activates it. TMPRSS2 helps in protein priming in SARS-CoV, supporting viral uptake [25]. ACE2 is being studied well in literature, involved in various pathways such as the ACE-RAGE pathway in diabetic patients, FOXO signaling, HIF signaling, longevity regulation, and renin-angiotensin pathways. TMPRSS2 is a receptor protein that is also involved in providing entry to the host but is not much studied and could have the potential to be targeted against various signaling pathways of coronavirus 2019, influenza A, and transcriptional dysregulation in various cancers like prostate cancer.

Diabetes and cancer are the major risk factors for the progression and prognosis of COVID-19 due to the abundance of expression of ACE2 in different organs [26, 27]. In addition, the anecdotal models of evidence evaluated that coronavirus unveils the variations in morbidity and mortality among sexes. Males are almost 3 times more prone to the virus than females [28]. The diversity of plants in the treatment of COVID-19 has shown interaction with other active enzymes involved in its pathogenic pathway.

Though certain medications have been used for COVID-19 patients, these synthetic drugs exhibit toxicity which may lead to hyperstimulation of cells and reduction in function [29]. For example, hydroxy-chloroquininone and 2-DG (2-deoxy-D-glucose) have been recently used to suppress COVID infection but they have been reported to have increased melanin, skin rashes, dizziness, ocular, and cardiac toxicity. Hence, there is an immediate and utmost requirement for an alternative that exhibits phytotherapeutic assistance to COVID-19 patients and synergistically improves the activity of an organism.

Natural herbs are being used for a century, with their development and production in the form of different formulations. In India, almost 70% population relies on using traditional medicines to cure many prevalent diseases [30]. They produce a wide range of bioactive compounds, with probable anti-microbial and anti-viral activities, etc., against a lot of agents. In our study, we have used two plants, *Piper longum* and *Ocimum sanctum*, for their prominent role as ACE2 and TRMPSS2 modulators. *Piper longum* or Pipali is generally used in houses for cooking purposes as a spice, and its medicinal role has been discussed in several studies. The plant has been a widely used remedy for several diseases like tuberculosis, gonorrhea, respiratory infections, immune-modulatory and anti-tumor activities, CNS depressant, anti-inflammatory, and anti-oxidative properties [31, 32]. *Piper longum* is a deciduous aromatic climber plant that has perennial roots and shrubs. These are monoecious where male and female flowers are present in separate structures on the same plant. This is mostly grown in India, Nepal, Sri Lanka, and the Philippines. *P. longum*, in general, comprises piperine, anthraglycosides, sterols, alkaloids, vicenin, and several other compounds [33]. *Ocimum sanctum*, Tulsi, is holy basil encompassing prominent importance in Indian Hindu Culture. Tulsi is one of the primogenital herbs of the family *Lamiaceae* and is found in almost every household because of its medicinal and nutritional properties. The ecological supremacy of Tulsi indicates their photosynthetic and pharmaceutical efficiency, as seen in various studies [34]. *O. sanctum* e is anti-viral, anti-bacterial, and anti-cancerous and treats respiratory problems and many other diseases [35]. They consist of extensive varieties of secondary metabolites like tannins, phenolic, alkaloids, and flavonoids, which support enhancing growth and immunity responses [36]. The present study is designed to observe the effect and efficacy of phytocompounds from selected herbs as ACE2 and TRMPSS2 inhibitors. To the best of our knowledge, there has been no study conducted so far. The proposed study is novel and has huge potential in a current pandemic.

Materials and Method

Receptor Preparation Using Protein Preparation Wizard and Receptor Grid Generation (PRIME)

3D structures of ACE2 and TMPRSS2 were downloaded from Protein Data Bank (ACE2 PDB ID: 1R4L and TMPRSS2 PDB ID: 7MEQ). These proteins were initially associated with one of their known inhibitors. The receptor proteins were prepared using the Prime

Module of Schrodinger Suite. The ligands were removed from both the receptors, and the final structure was cleaned and saved in PDB format. All the proteins were prepared by removing water, hydrogen, and existing lead components like ions. Chain A from the proteins was deleted, and respected force fields and charges were added. The grid was formed around the active sites of the known inhibitors using the Receptor Grid Generation tool with a dimension of 10 Armstrong [37].

Ligand Molecule Preparation

For the discovery of any drug or effective compound, in silico studies provide crucial clues. To combat cost and time, in silico approach becomes the foremost choice in research. For a molecule to be a drug, it has to follow Lipinski's rule which states five categories to be verified, also known as a "rule of thumb." These include that a chemical entity must have a molecular weight less than or equal to 500 Da, 5 or fewer hydrogen bonds donors, 10 or fewer hydrogen acceptors, MLOGP value less than and equal to 4.15, and molecular refractivity has to be in between 30 and 140 [38]. The ligands were downloaded from IMPPAT (Indian Medicinal Plants, Phytochemistry, and Therapeutics) in SDF format. 2-DG and nafamostat were taken as control drugs. The ligands were prepared using the Ligprep module of the Schrodinger suite. The ligands were neutralized and desalted, and Epik ionization was provided.

Molecular Docking and Visualization

Molecular docking predicts the binding energies based on the affinity of the ligand with the receptor. The prepared proteins and ligands were used for molecular docking studies. Both of them were processed and docked in the ligand docking tab. The zip document of the grid file of protein was uploaded against the ligand output of the file. Extra precision (XP) docking was done to eliminate respective penalties and errors. After docking, the structures were visualized in Maestro software. Top scoring outputs processed were analyzed in the ligand interaction diagram to check interaction and amino acid involved [31, 39].

SwissADME

The SwissADME is a freely accessible web tool (http://www.swissadme.ch/) and provides an easy way to analyze results in a computer-aided drug designing platform. The web-based tool provides pharmacokinetics data, physiochemical properties, lipophilicity, water solubility, and drug-likeness, and illustrates the molecule in a boiled egg, showing whether the ligand can cross the blood–brain barrier and gastrointestinal tract [32, 40]. The selected phytomolecules were analyzed for their ADME profiling.

Enrichment Analysis for Specific Cellular Pathways

To classify cellular pathways which were enriching host factors interacting with coronavirus spike protein, KEGG (https://www.genome.jp/kegg/pathway.html) annotations were retrieved [41]. To evaluate combining interactive information from both receptor proteins, i.e., ACE2 and TMPRSS2, pathways involved in different functions were being implied.

Interaction between Proteins by STRING Network Analysis

Search Tool for Retrieval of Interacting Genes (STRING) (https://string-db.org/) Database predicts and integrates protein–protein interaction to identify functional relationships and interactions between proteins. To seek potential interactions between genes involved in SARS-CoV, the STRING tool was employed. STRING provided a platform that postulated several nodes, edges, average node degrees, protein–protein interaction (PPI) enrichment *p*-values, and average and local clustering coefficient. STRING provided possible biological processes, molecular function, and cellular components of candidate genes studied [42].

Bioactivity Score Analysis Using Molinspiration Tool

Molinspiration (http://www.molinspiration.com/cgi-bin/properties) predicts the drug resemblance properties of the compound dependent on various descriptors. Drugs entering the body should tie to an organic molecule to communicate its movement. Along their path, the bioactivity of compounds was anticipated by utilizing the Molinspiration tool which gave a bioactivity score of the phytocompound against the human receptors like GPCRs, ionic channels, kinases, various receptors, proteases, and proteins. A complex is considered to be dynamic if the bioactivity score is more than 0.0, modestly dynamic if somewhere in the range of − 5.0 and 0.0, and idle if under − 5.0 [43].

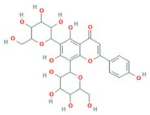
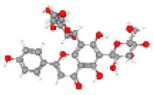
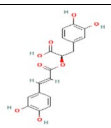
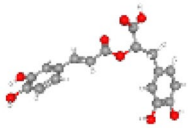
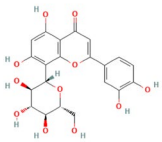
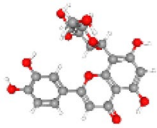
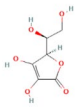
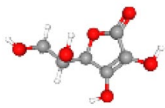
Results and Discussion

We first conducted molecular docking to predict the probable protein–ligand interactions by minimizing the energy of the ligands and calculating their binding energies. Binding of ligand to respective receptor protein causes an inhibition of ligand toward enzyme, which results in a demonstration of the feasibility of a biochemical reaction. Docking algorithms utilize inhibitory and activator properties of the ligand with receptor protein and form a relationship between the drug's structure and cytotoxicity.

We selected approximately a hundred phytocompounds from *Piper longum* and *Ocimum sanctum* using the IMPPAT database. ACE2 and TMPRSS2 were docked with all phytostructures. The docking score of significant interactions (~ 15) is shown in Table 1. However, vicenin 2, rosmarinic acid, and orientin were found to be most efficient. For ACE2, we obtained the highest binding energy of − 11.755 kcal/mol with vicenin 2. Also, for TMPRSS2, vicenin has shown the binding energy of − 7.913 kcal/mol. 2-DG (an ACE2 inhibitor) and nafamostat (a TMPRSS2 inhibitor) were taken as controls for both the targets as it has been currently used and have shown good efficacy as synthetic drugs against SARS-CoV infection. The binding energy of 2-DG with ACE2 was found to be − 6.05 kcal/mol, while for TMPRSS2, nafamostat binding energy was − 2.188 kcal/mol. The interaction poses of vicenin 2, rosmarinic acid, and orientin with ACE2 and TMPRSS2 are shown in Figs. 1 and 2, respectively. The amino acids shared by the selected phytocompounds and controls were found to be common suggesting the involvement of a similar biological mechanism.

For ACE2, the binding activities were found to be in the following order: vicenin 2 > rosmarinic acid > orientin > L-ascorbic acid > 2,6-diaminohexanoic acid > luteolin 7-O-glucuronide > lignans machilin F > 2,4-dihydroxycinnamic acid > 7,3′,4′,5′-tetrahydroxyflavone > luteolin > oleic

Table 1 Molecular docking analysis of the ACE2 and TMPRSS2 receptor with selected phytocompounds

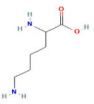
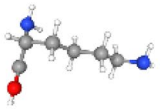
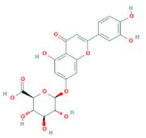
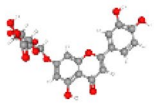
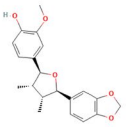
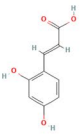
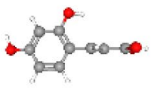
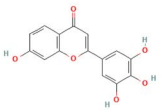
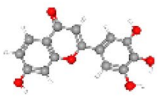
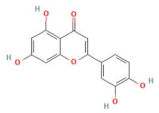
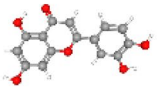
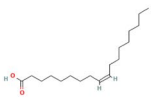
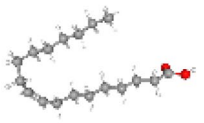
Phyto-compounds	Binding affinity (kcal/mol)	2D structures	3D structures
Docking with ACE2			
Vicenin 2	−11.755		
Rosmarinic acid	−10.18		
Orientin	−10.132		
L-ascorbic acid	−8.036		

acid > 2-coumarinate > 1-(4-hydroxy-3-methoxyphenyl)-1,2,3-tris(4-allyl-2-methoxyphenoxy) propane > palmitic acid > 2-deoxy-glucose, whereas for TMPRSS2, the binding activities were as follows:vicenin2 > rosmarinicacid > luteolin7-O-glucuronide > orientin > luteolin > eupatin > MLS000877024 > esculetin > 1-(4-hydroxy-3-methoxyphenyl)-1,2,3-tris(4-allyl-2-methoxyphenoxy) propane > molludistin > lignans machilin F > nevadensin > apigenin > nafamostat.

Our in silico molecular docking studies suggested the significantly higher binding activities of selected phytocompounds with ACE2 and TMPRSS2 as compared to the present-day synthetic inhibitors used in the study which shows the therapeutic relevance of *Piper longum* and *Ocimum sanctum* in combating COVID complications.

To further determine the drug-likeliness properties of selected phytocompounds, ADME analysis was done. Lipinski et al. formulated certain parameters by defining their physiochemical ranges to be an oral drug, called their drug-likeness. Rosmarinic acid showed promising results with control 2-DG and nafamostat against ACE2 and TMPRSS2. The physiochemical properties like molecular weight, number of hydrogen bonds acceptors and donors, and number of rotatable bonds show a great similarity with 2-DG and nafamostat. The lipophilicity results of rosmarinic acid showed positive values which demonstrates the greater affinity toward the lipid environment. In comparison to controls, 2-DG has less affinity and nafamostat confers better affinity in a lipid environment. In addition, rosmarinic acid has 0 violations and almost the same bioavailability score of 0.55 in comparison with

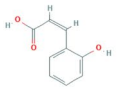
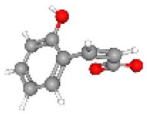
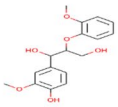
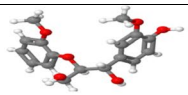
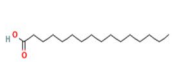
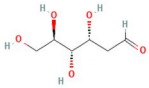
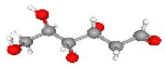
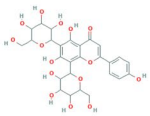
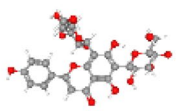
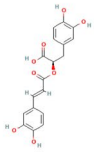
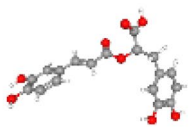
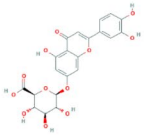
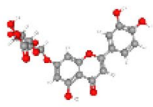
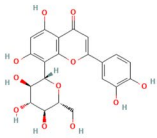
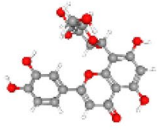
Table 1 (continued)

2,6-Diaminohexanoic acid	−7.823		
Luteolin 7-O-glucuronide	−7.278		
Lignans machilin F	−7.198		
2,4-Dihydroxycinnamic acid	−6.938		
7,3',4',5'-Tetrahydroxyflavone	−6.707		
Luteolin	−6.68		
Oleic acid	−6.617		

2-DG and nafamostat. They all had features to be a candidate drug molecule; however, rosmarinic acid is found to be a better option. The absorption and bioavailability of the selected compounds can be improved in the future with various formulations (Table 2).

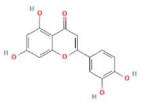
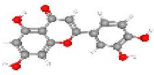
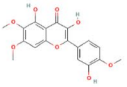
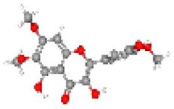
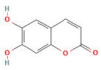
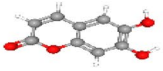
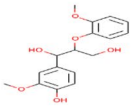
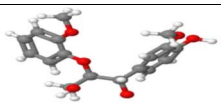
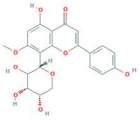
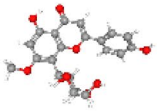
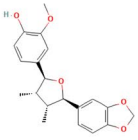
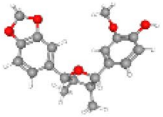
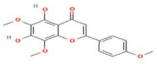
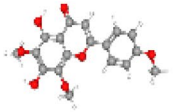
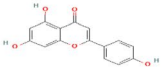
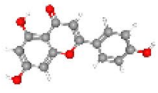
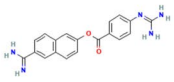
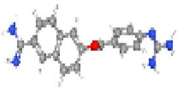
We next predicted the target network to observe the interactors of ACE2 and TMPRSS2 using STRING. ACE2 was found to be interacting with several proteins as shown in Fig. 3. These proteins were found to engage in various biological processes such as in renin-angiotensin regulation of aldosterone production, GPCR-signaling pathways, cell development, brain renin-angiotensin system, angiotensin maturation, cell growth involved in cardiac muscle, amyloid-beta metabolic process, and vasodilation.

Table 1 (continued)

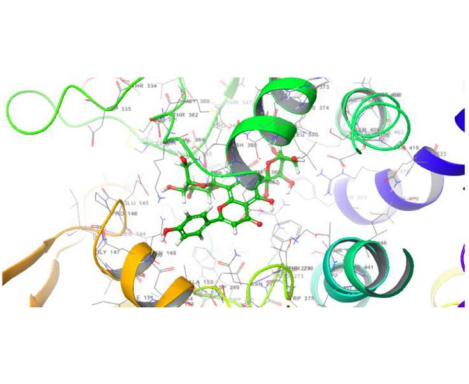
2-Coumarinate	−6.233		
1-(4-Hydroxy-3-Methoxyphenyl)-1,2,3-Tris(4-Allyl-2-Methoxyphenoxy) Propane	−6.041		
Palmitic acid	−6.016		
2-DG	**−6.05**		
Docking with TMPRSS2			
Vicenin 2	−7.913		
Rosmarinic acid	−7.137		
Luteolin 7-O-glucuronide	−6.866		
Orientin	−6.523		

Angiotensin type II receptor activity, dipeptidyl-peptidase activity, aminopeptidase, carboxypeptidase, and exopeptidase activity were their majorly concerned molecular functions. On the other hand, TMPRSS2 was observed to be interacting with proteins majorly engaged in the regulation of epithelial cell proliferation, prostate gland development and their growth, protein kinase B signaling, regulation of insulin-like growth factor receptor signaling pathway, and androgen receptor signaling pathway. The foremost

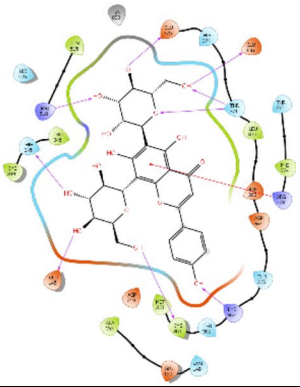
Table 1 (continued)

Luteolin	−6.404		
Eupatin	−6.329		
Esculetin	−5.856		
1-(4-Hydroxy-3-Methoxyphenyl)-1,2,3-Tris(4-Allyl-2-Methoxyphenoxy) Propane	−5.667		
Molludistin	−5.317		
Lignans machilin F	−5.235		
Nevadensin	−5.172		
Apigenin	−5.0		
Nafamostat	**−2.188**		

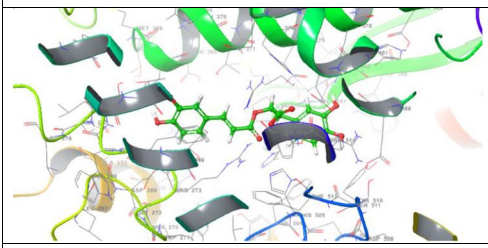
molecular function of TMPRSS2 was found to have nuclear receptor activity, serine-type endopeptidase activity, DNA-binding transcription activator activity, and RNA polymerase II-specific activity.



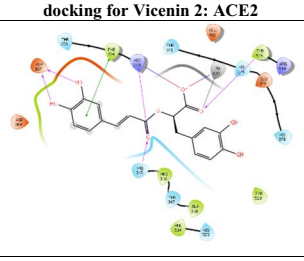
(2a) Donor: acceptor surface for Vicenin 2: ACE2



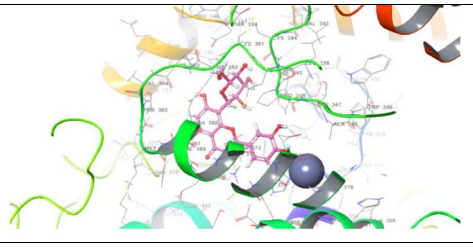
(2b) Possible types of interactions obtained from docking for Vicenin 2: ACE2



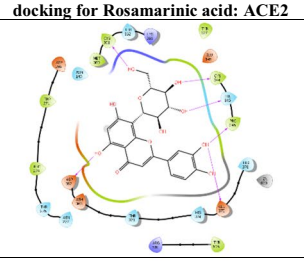
(2c) Donor: acceptor surface for Rosamarinic acid: ACE2



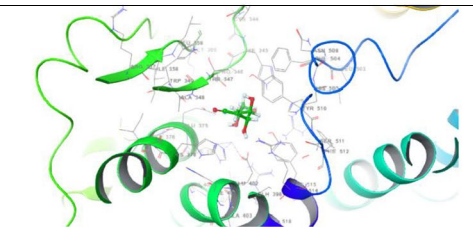
(2d) Possible types of interactions obtained from docking for Rosamarinic acid: ACE2



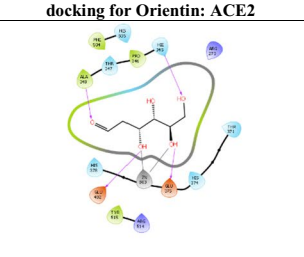
(2e) Donor: acceptor surface for Orientin: ACE2



(2f) Possible types of interactions obtained from docking for Orientin: ACE2

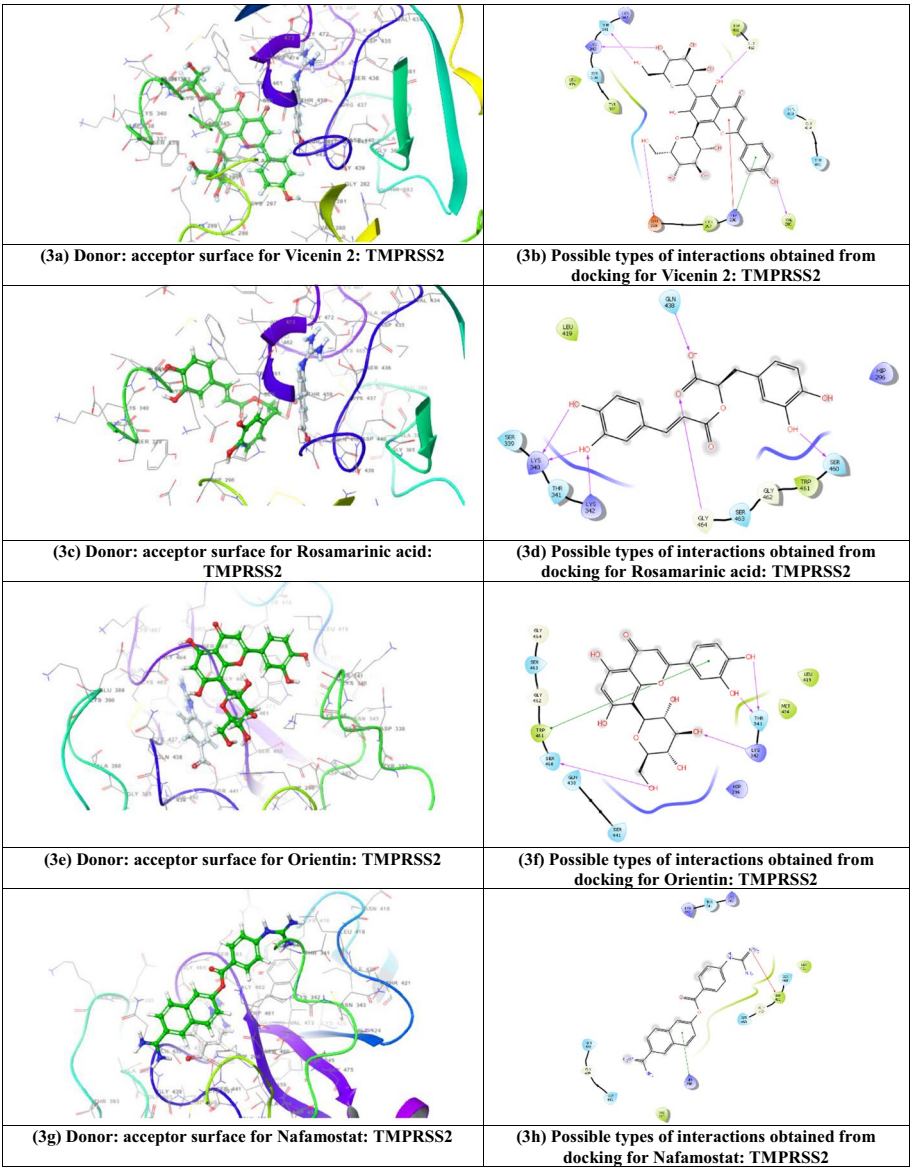


(2g) Donor: acceptor surface for 2-DG: ACE2



(2h) Possible types of interactions obtained from docking for 2-DG: ACE2

Fig. 1 Donor:acceptor surface and possible interactions obtained from docking for ACE2 with vicenin 2, rosmarinic acid, orientin, and control 2-DG. **a** Donor:acceptor surface for vicenin 2: ACE2. **b** Possible types of interactions obtained from docking for vicenin 2:ACE2. **c** Donor:acceptor surface for rosamarinic acid: ACE2. **d** Possible types of interactions obtained from docking for rosamarinic acid:ACE2. **e** Donor:acceptor surface for orientin:ACE2. **f** Possible types of interactions obtained from docking for orientin:ACE2. **g** Donor:acceptor surface for 2-DG:ACE2. **h** Possible types of interactions obtained from docking for 2-DG:ACE2



(3a) Donor: acceptor surface for Vicenin 2: TMPRSS2

(3b) Possible types of interactions obtained from docking for Vicenin 2: TMPRSS2

(3c) Donor: acceptor surface for Rosamarinic acid: TMPRSS2

(3d) Possible types of interactions obtained from docking for Rosamarinic acid: TMPRSS2

(3e) Donor: acceptor surface for Orientin: TMPRSS2

(3f) Possible types of interactions obtained from docking for Orientin: TMPRSS2

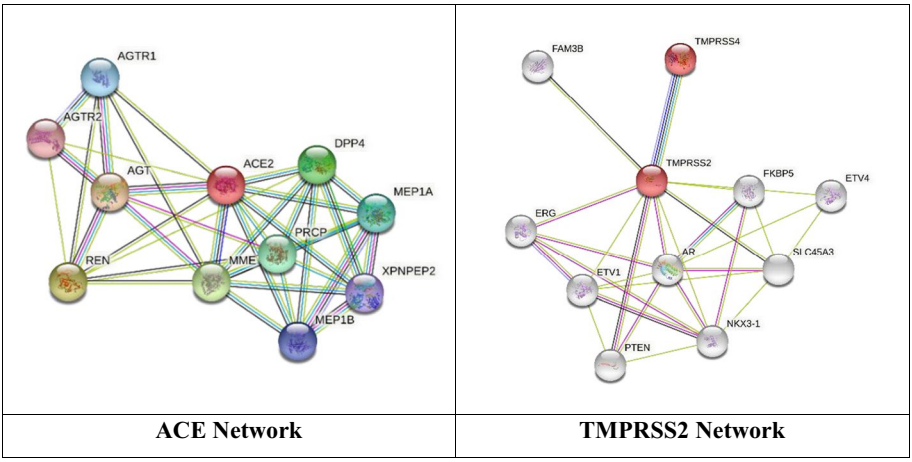
(3g) Donor: acceptor surface for Nafamostat: TMPRSS2

(3h) Possible types of interactions obtained from docking for Nafamostat: TMPRSS2

Fig. 2 Donor:acceptor surface and possible interactions obtained from docking for TMPRSS2 with vicenin 2, rosmarinic acid, orientin, and control nafamostat. **a** Donor:acceptor surface for vicenin 2:TMPRSS2. **b** Possible types of interactions obtained from docking for vicenin 2:TMPRSS2. **c** Donor:acceptor surface for rosamarinic acid:TMPRSS2. **d** Possible types of interactions obtained from docking for rosamarinic acid:TMPRSS2. **e** Donor:acceptor surface for orientin:TMPRSS2. **f** Possible types of interactions obtained from docking for orientin:TMPRSS2. **g** Donor:acceptor surface for nafamostat:TMPRSS2. **h** Possible types of interactions obtained from docking for nafamostat:TMPRSS2

Table 2 Drug-likeliness properties of phytoconstituents from *Piper longum* and *Ocimum sanctum* by SWISS ADME data

Drug-likeness properties	Vicenin 2	Rosmarinic acid	Orientin	2-DG	Nafamostat
Physiochemical properties					
Molecular weight (gm/mol)	594.52	360.31	448.38	164.16	347.37
Number of H-bond acceptors	15	8	11	5	4
Number of H-bond donors	11	5	8	4	4
Number of rotatable bonds	5	7	3	5	5
Molar refractivity	139.23	91.40	108.63	35.80	101/24
Topological polar surface area TPSA (A^2)	271.20	144.52	201.28	97.99	140.57
Lipophilicity					
Log Po/w (iLOGP)	1.27	1.17	1.27	− 0.04	1.50
Log Po/w (XLOGP3)	− 2.26	2.36	− 0.15	− 2.88	2.03
Log Po/w (WLOGP)	− 3.04	1.65	− 0.53	− 2.35	2.25
Log Po/w (MLOGP)	− 4.51	0.90	− 2.51	− 2.10	2.96
Log Po/w (SILICOS-IT)	− 1.80	1.50	− 0.14	− 0.93	1.94
Consensus Log Po/w	− 2.07	1.52	− 0.41	− 1.66	2.14
Drug-likeness					
Lipinski	3 violations	0 violation	2 violations	0 violation	0 violation
Bioavailability score	0.17	0.56	0.17	0.55	0.55
Synthetic accessibility (SA)	6.40	3.38	5.17	2.97	2.58
Pharmacokinetics					
GI absorption	Low	Low	Low	Low	Low
BBB permeant	No	No	No	No	No
P-gp substrate	No	No	No	No	No
Log Kp (skin permeation, cm/s)	− 11.53	− 6.82	− 9.14	− 9.35	− 6.98
Water solubility					
Log S (ESOL)	− 2.05	− 2.17	− 2.70	− 2.05	− 5.35
Solubility (mg/mL)	3.19e + 02 mg/ml; 5.36e–01 mol/l	2.41e + 00 mg/ml; 6.70e–03 mol/l	7.32e + 00 mg/ml; 1.63e–02 mol/l	5.25e + 00 mg/ml; 8.83e–03 mol/l	1.55e–03 mg/ml; 4.46e-06 mol/l



ACE Network | **TMPRSS2 Network**

Fig. 3 Network formation of ACE2 and TMPRSS2 using STRING

KEGG analysis was conducted to further analyze the biological relevance of the identified target and the possible involvement of ACE2 and TMPRSS2. A total of 20 key targets including AR, PTEN, and ERG were identified through PPI network analysis. These targets were mainly focused on the biological processes with ACE2 and TMPRSS2. The KEGG enrichment manifested signaling pathways that were closely related to COVID-19 and renin-angiotensin system, as shown in Figs. 4 and 5. Furthermore, in the COVID-19 pathway, TMPRSS2 and NRP1 are involved in the entry of spike protein via membrane fusion or endocytosis. Another pathway is involved in the renin-angiotensin system wherein Angiotensin (1-7) and Angiotensin II are entered in the host cell by MAS1 and AT1R and further facilitated by ADAM17, TNFR, EGFR, and TLR2/4.

Lastly, we predicted bioactivity scores to identify the potency of phytomolecules studied (Table 3). Our result showed that rosmarinic acid and orientin might essentially serve as an interface with ACE2 and TMPRSS2 as they acted as enzyme inhibitors. Bioactivity scores of herbal compounds and controls were comparable indicating that these phytocompounds might be utilized as an alternative and in improving treatment to COVID.

The prediction of possible targets leads to mechanistic pathways involved in coronavirus integration into the human body and associated chronic diseases. ACE2 and TMPRSS2 facilitate the virus entry into the human genome. The ligands have shown explicit findings in recent times and have been proposed to show major impacts on coronavirus. Molecular docking of the compounds with ligands has provided good binding energy, and the study is projected to assess ligands in vitro analysis. Out of all compounds, rosmarinic acid and orientin have revealed effective energies and follow Lipinski's rule to have drug-likeness properties. Downstream to molecular targets, these proteins are also associated with other molecular functions like cell signaling, apoptosis, DNA-binding transcription activator activity, and amyloid-beta metabolic process, which eventually are targeted when dysregulated. We propose phytoconstituents of *Piper longum* and *Ocimum sanctum* as ACE2 and TMRSS2 inhibitors: strategies to

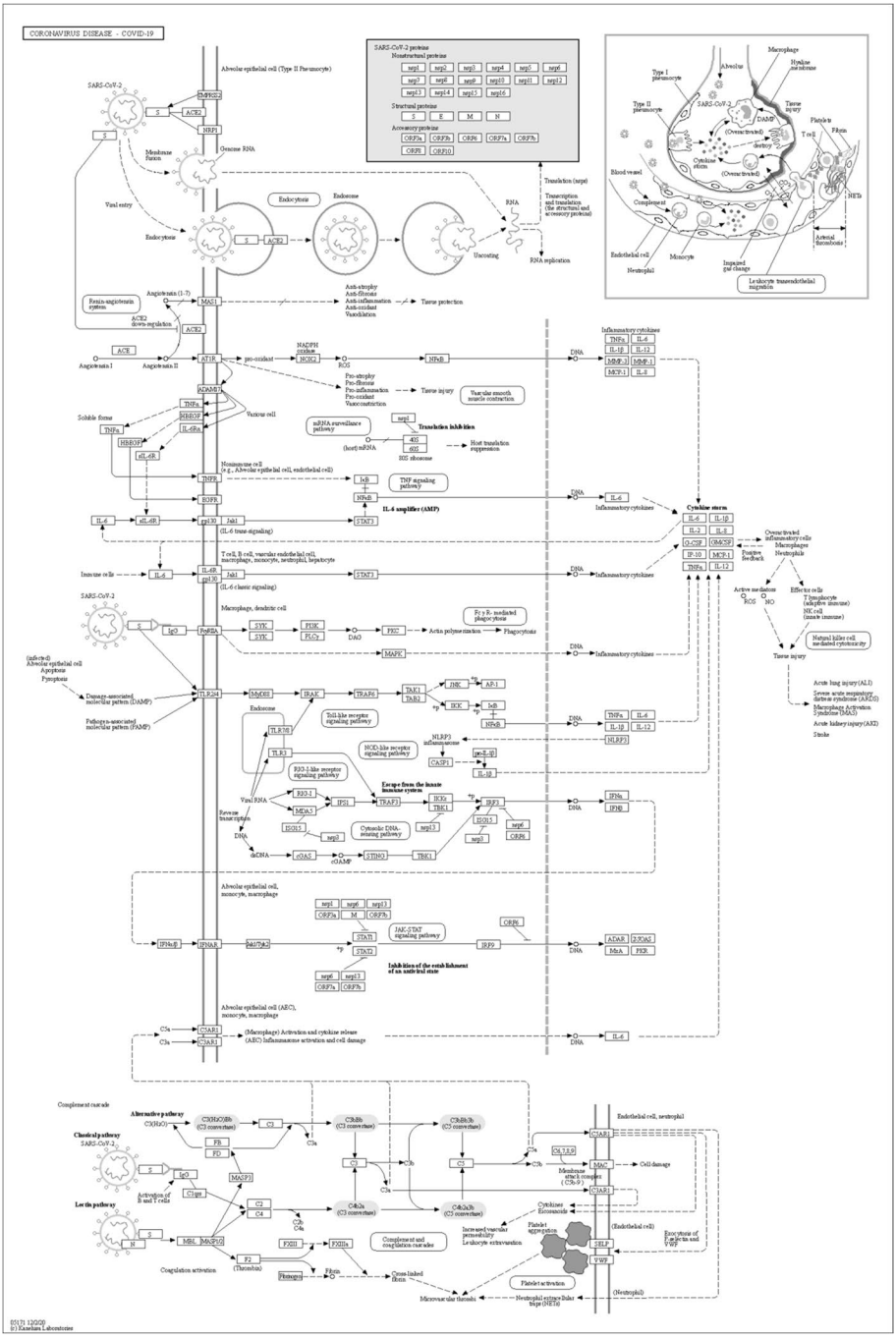


Fig. 4 KEGG analysis of ACE2 involved in COVID-19

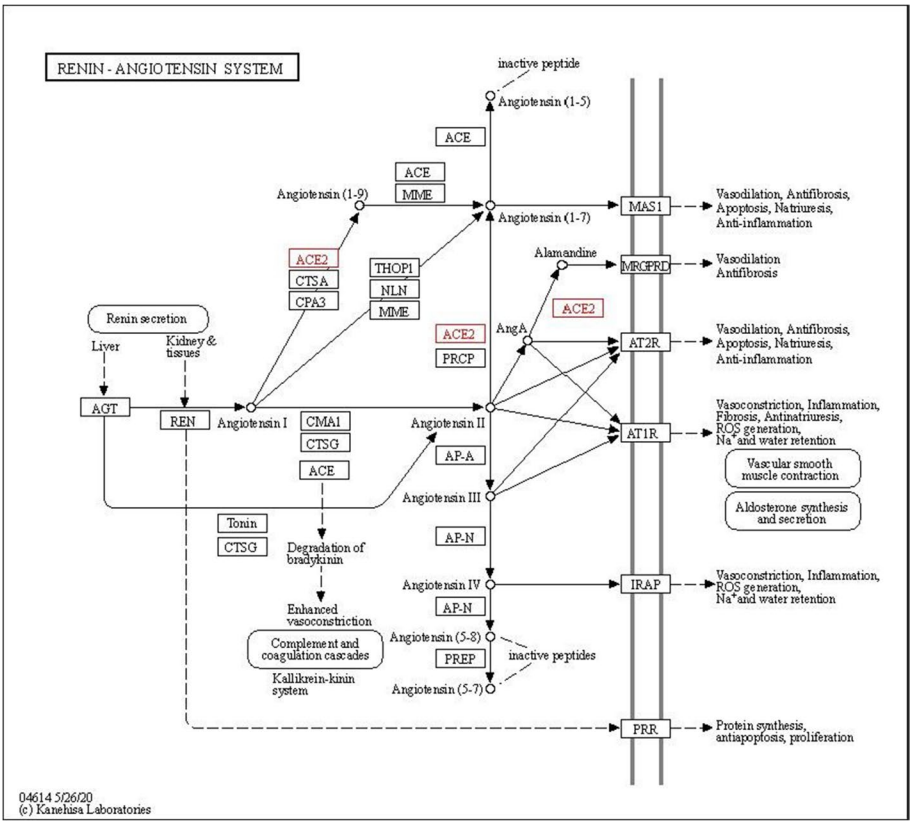


Fig. 5 KEGG analysis of ACE2 involved in the renin-angiotensin system

Table 3 Summary of predicted Molinspiration bioactivity score for vicenin 2, rosmarinic acid, and orientin in comparison to 2-DG

Bioactivity	Vicenin 2	Rosmarinic acid	Orientin	2-DG	Nafamostat
GPCR ligand	0.05	0.17	0.12	−0.62	0.28
Ion channel modulator	−0.41	−0.08	−0.14	0.07	0.14
Kinase inhibitor	−0.06	−1.18	0/20	−1.00	−0.03
Nuclear receptor ligand	−0.03	0.57	0.20	−0.76	−0.16
Protease inhibitor	−0.03	0.15	0.01	−0.17	0.57
Enzyme inhibitor	0.20	0.24	0.45	0.31	0.19

combat COVID-19 (Fig. 6). However, such understandings need further approval utilizing distinctive in vitro experiments and molecular dynamics simulation.

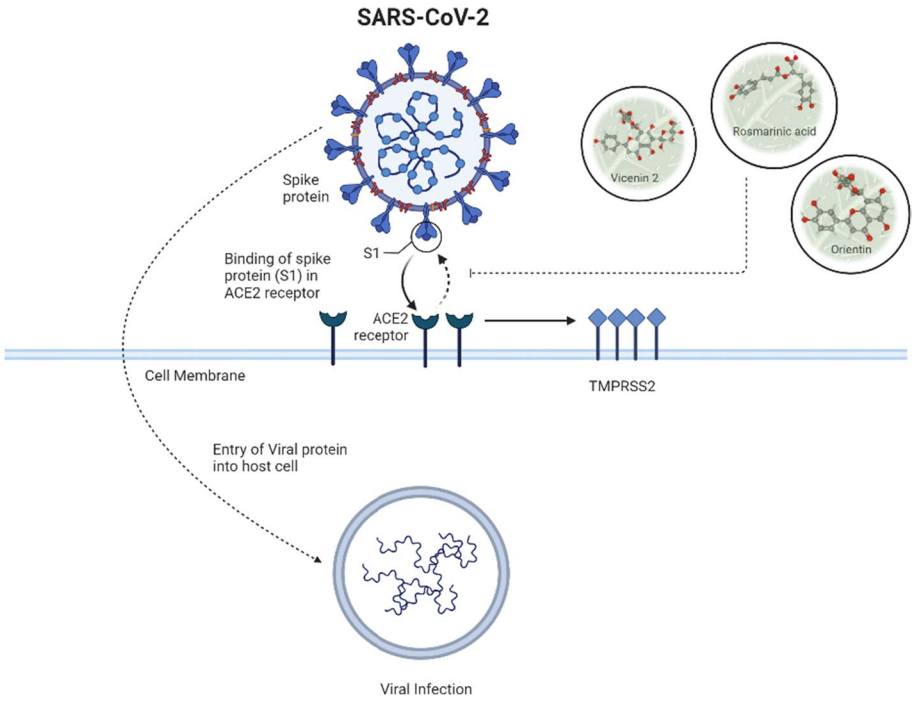


Fig. 6 Proposed mechanism of action of *Piper longum* and *Ocimum sanctum* phytocompounds

Conclusion

The traditional use of Indian medicinal plants is believed to be a huge foundation for the treatment of diseases. For this study, we have investigated the potential of some phytocompounds which are being extracted from the most common medicinal plants: *Piper longum* and *Ocimum sanctum.* The results are projected to seize the attention of scientists in the area of drug discovery against COVID-19 for which no specific and successful drug has been discovered with natural phytocompounds. The approach is simply using natural bioactive components, and the same investigation can also be done by various available medicinal herbs. In addition, our study is extended to see the effects of other diseases contributing to the progression of the coronavirus.

Acknowledgements The authors acknowledge Jaypee Institute of Information Technology, Noida, for providing the entire infrastructure to complete this project.

Author Contribution Both the authors equally contributed.

Funding This study did not require any specific funding support. This work was supported by the Institutional fund of the host organization.

Availability of Data and Material In silico data and material are present in the manuscript.

Code Availability No code applicable.

Declarations

Ethics Approval No ethical approval is required in the study.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

References

1. Mira Bosso TAT, Mohamed Abu-Farha, Muath Alanbaei, Jehad Abubaker, Fahd Al-Mulla. The two faces of ACE2: The role of ACE2 receptor and its polymorphisms in hypertension and COVID-19. Molecular Therapy - Methods and Clinical Development. 2020;18:321–7.
2. Beadling, C., & Slifka, M. K. (2004). How do viral infections predispose patients to bacterial infections? *Current Opinion in Infectious Diseases, 17*(3), 185–191.
3. Guo, Y. R., Cao, Q. D., Hong, Z. S., Tan, Y. Y., Chen, S. D., Jin, H. J., et al. (2020). The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – An update on the status. *Military Medical Research, 7*(1), 11.
4. Graham, R. L., Donaldson, E. F., & Baric, R. S. (2013). A decade after SARS: Strategies for controlling emerging coronaviruses. *Nature Reviews Microbiology, 11*(12), 836–848.
5. Li, Y. C. (2003). Vitamin D regulation of the renin-angiotensin system. *Journal of Cellular Biochemistry, 88*(2), 327–331.
6. Cleland, J. G. F., Henderson, E., McLenachan, J., Findlay, I. N., & Dargie, H. J. (1991). Effect of Captopril, an angiotensin-converting enzyme inhibitor, in patients with angina pectoris and heart failure. *Journal of the American College of Cardiology., 17*(3), 733–739.
7. Imai, Y., Kuba, K., & Penninger, J. M. (2008). The discovery of angiotensin-converting enzyme 2 and its role in acute lung injury in mice. *Experimental Physiology, 93*(5), 543–548.
8. Chiknas, S. G. (1979). A liquid chromatography-assisted assay for angiotensin-converting enzyme (peptidyl dipeptidase) in serum. *Clinical Chemistry., 25*(7), 1259–1262.
9. Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., et al. (2000). A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. *Circulation Research, 87*(5), E1-9.
10. Valera, P., Letuka, P., Mathenjwa, N., Ntusi, N. Angiotensin converting enzyme inhibitors, angiotensin receptor blockers, mineralocorticoid receptor antagonists and SARS-CoV-2 infection. SA Heart. 2020;17.
11. Lange, C. W. J., Auw-Haedrich, C., Schlecht, A., Boneva, S., Lapp, T., et al. (2020). Expression of the COVID-19 receptor ACE2 in the humanconjunctiva. *Journal of Medical Virology., 92*, 2081–2086.
12. Kuba, K., Imai, Y., Rao, S., Gao, H., Guo, F., Guan, B., et al. (2005). A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nature Medicine, 11*(8), 875–879.
13. Velavan, T. P., & Meyer, C. G. (2020). The COVID-19 epidemic. *Tropical Medicine & International Health, 25*(3), 278–280.
14. Glowacka, I., Bertram, S., Müller, M. A., Allen, P., Soilleux, E., Pfefferle, S., et al. (2011). Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. *Journal of Virology, 85*(9), 4122–4134.
15. Unger, T., Li, J. The role of the renin-angiotensin-aldosterone system in heart failure. Journal of the renin-angiotensin-aldosterone system : JRAAS. 2004;5 Suppl 1:S7–10.
16. Turner, A. J., Hiscox, J. A., & Hooper, N. M. (2004). ACE2: From vasopeptidase to SARS virus receptor. *Trends in Pharmacological Sciences, 25*(6), 291–294.
17. Keiji Kuba YI, Josef M. Penninger. Multiple functions of angiotensin-converting enzyme 2 and its relevance in cardiovascular diseases. Circulation Journal. 2013;77.
18. Zhang, H., Penninger, J. M., Li, Y., Zhong, N., & Slutsky, A. S. (2020). Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: Molecular mechanisms and potential therapeutic target. *Intensive Care Medicine, 46*(4), 586–590.
19. Verdecchia, P., Cavallini, C., Spanevello, A., & Angeli, F. (2020). The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. *European Journal of Internal Medicine, 76*, 14–20.
20. Transmembrane protease serine 2 (TMPRSS2). Science-Business eXchange. 2014;7(39):1155.
21. Colman, P. M., & Lawrence, M. C. (2003). The structural biology of type I viral membrane fusion. *Nature Reviews Molecular Cell Biology., 4*(4), 309–319.

22. Alcott, B., Wu, Z., O'Shaughnessy, B., & Karatekin, E. (2015). Viral membrane fusion at single pore resolution. *Biophysical Journal., 108*, 406a.
23. Belouzard, S., Millet, J. K., Licitra, B. N., & Whittaker, G. R. (2012). Mechanisms of coronavirus cell entry mediated by the viral spike protein. *Viruses, 4*(6), 1011–1033.
24. Simmons, G., Zmora, P., Gierer, S., Heurich, A., & Pöhlmann, S. (2013). Proteolytic activation of the SARS-coronavirus spike protein: Cutting enzymes at the cutting edge of antiviral research. *Antiviral Research, 100*(3), 605–614.
25. Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell, 181*(2), 271–80.e8.
26. Bhowmick, N. A., Oft, J., Dorff, T., Pal, S., Agarwal, N., Figlin, R. A., et al. (2020). COVID-19 and androgen-targeted therapy for prostate cancer patients. *Endocrine-Related Cancer, 27*(9), R281–R292.
27. Zaki, N., Alashwal, H., & Ibrahim, S. (2020). Association of hypertension, diabetes, stroke, cancer, kidney disease, and high-cholesterol with COVID-19 disease severity and fatality: A systematic review. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews, 14*(5), 1133–1142.
28. Peckham, H., de Gruijter, N. M., Raine, C., Radziszewska, A., Ciurtin, C., Wedderburn, L. R., et al. (2020). Male sex identified by global COVID-19 meta-analysis as a risk factor for death and ITU admission. *Nature Communications, 11*(1), 6317.
29. Creagh S, Warden D, Latif MA, Paydar A. The new classes of synthetic illicit drugs can significantly harm the brain: A neuro imaging perspective with full review of MRI findings. Clin Radiol Imaging J. 2018;2(1).
30. Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology, 4*, 177.
31. Friesner, R. A., Murphy, R. B., Repasky, M. P., Frye, L. L., Greenwood, J. R., Halgren, T. A., et al. (2006). Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein–ligand complexes. *Journal of Medicinal Chemistry., 49*(21), 6177–6196.
32. Daina, A., & Zoete, V. (2016). A BOILED-Egg to predict gastrointestinal absorption and brain penetration of small molecules. *ChemMedChem, 11*(11), 1117–1121.
33. Ghoshal, S., & Lakshmi, V. (2002). Potential antiamoebic property of the roots of *Piper longum* Linn. *Phytotherapy research: PTR., 16*, 689–691.
34. Batra, N. G., Kumari, N., & Sharma, V. (2016). Photosynthetic performance of Ocimum sanctum morphotypes in a semiarid region. *Journal of Herbs, Spices & Medicinal Plants., 22*(3), 211–224.
35. Borah r BSP. Tulsi (Ocimum sanctum), excellent source of phytochemicals. International Journal of Environment, Agriculture and Biotechnology (IJEAB).3(5).
36. G.I. Ameh CSE. Phytochemical constituents of some nigerian plants. African Journals Online. 2010;8(1).
37. Jacobson MP, Pincus DL, Rapp CS, Day TJF, Honig B, Shaw DE, et al. A hierarchical approach to all-atom protein loop prediction. Proteins: Structure, Function, and Bioinformatics. 2004;55(2):351–67.
38. Lipinski, C. A. (2004). Lead- and drug-like compounds: The rule-of-five revolution. *Drug Discovery Today: Technologies, 1*(4), 337–341.
39. Yuriev, E., Agostino, M., & Ramsland, P. A. (2011). Challenges and advances in computational docking: 2009 in review. *Journal of Molecular Recognition., 24*(2), 149–164.
40. Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Science and Reports, 7*, 42717.
41. Dennis G, Jr., Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, et al. DAVID: Database for annotation, visualization, and integrated discovery. (1465–6906 (Print)).
42. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, et al. STRING v10: Protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res. 2015;43(Database issue):D447–52.
43. Tariq, M., Sirajuddin, M., Ali, S., Khalid, N., Tahir, M. N., Khan, H., et al. (2016). Pharmacological investigations and Petra/Osiris/Molinspiration (POM) analyses of newly synthesized potentially bioactive organotin(IV) carboxylates. *Journal of Photochemistry and Photobiology B: Biology, 158*, 174–183.