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Costunolide and Lupeol reinforce IRF3 gene activity in human immune response against COVID-19

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Abstract: Coronavirus disease 2019 (COVID-19), caused by the SARS-CoV-2 virus strain, is a significant threat worldwide due to its fast spreading among people. Currently, many vaccines have been used for prevention and to improve the immune system of people. Still, these vaccines are not prevented enough, and many severe side effects exist, including death in many patients. In this study, firstly, we attempted to identify the target gene responsible for driving the immune response against viruses through network and functional analyses; secondly, potential active components present in the ingredients of Kabasura Kudineer (15 herbal plants components) were identified, and its effect against COVID-19; thirdly, the transcription factor (IRF3) and active compounds of Kabasura Kudineer were involved in virtual screening. Pharmacokinetic properties were compared after predicting the ADMET values of Costunolide, Lupeol, Vazegepant and Methylprednisolone. The overall work revealed that the selected active compounds increase the transcription factor enhancement regarding the immune response.

Keywords: COVID-19; Kabasura Kudineer; IRF3; immune response; ADMET; vaccines; virtual screening.

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Biographical notes: Y. Fasila has completed her MSc in Bioinformatics from Bharathiar University. She did her project work under the Guidance of Jayaprakash Chinnappan. This paper is purely based on her project work.

Jayaprakash Chinnappan has obtained BSc in Animal Science and Biotechnology, MSc and PhD in Bioinformatics, MBA in Human Resources. Currently working as an Assistant Professor in Bharathiar University.

1 Introduction

In the second week of December 2019, an unknown virus infection was identified in very few native fish and wild animal markets in the urban center of Wuhan, Hubei province in

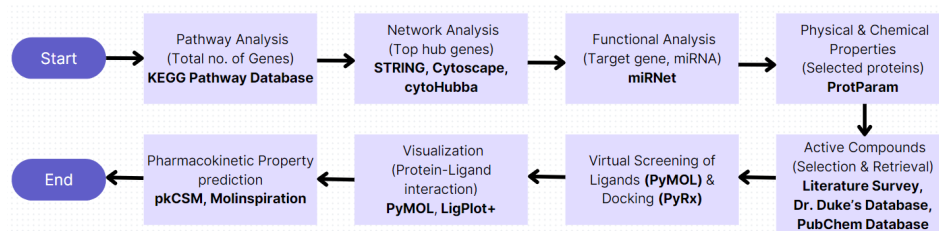
China (Swed et al., 2022). The Chinese Center for Sickness Management and bar (CCDC) knows this infection as a distinctive coronavirus infection. On January 7, 2020, and February 11, 2020, the World Health Organization (WHO) proclaimed a replacement name for the infectious sickness communicable disease as 2019-new coronavirus disease (2019-nCoV and currently observed as COVID-19). COVID-19 is caused by infection due to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus strain. Coronaviruses belong to the family Coronaviridae (order *Nidovirales*) and embrace viruses with a single-strand, positive-sense RNA order of about 26–32 kilobases in size (Yousefi and Eslami, 2022). The Coronaviridae family contains five dominant genera: Alpha-corona virus (alpha CoV), Beta-corona virus (beta CoV), Delta-corona virus (delta CoV), Gamma-corona virus (gamma CoV), and recently, Omicron variant. Coronaviruses are regarded as spike glycoproteins due to the presence of spikes pointing outwards to their envelope. The structure of SARS-CoV-2 has four structural proteins S (spike), E (envelope), M (membrane), and N (nucleocapsid) macromolecules (Malone et al., 2022).

COVID-19 will affect the upper respiratory tract (sinuses, nose, and throat) and additionally the lower respiratory tract (windpipe and lungs) (Harrison et al., 2020). The lung area unit is affected by SARS-CoV-2 due to the virus accessing the host cells via angiotensin-converting enzyme 2 (ACE2) (Verdecchia et al., 2020). The spike protein in the virus is meant to connect with ACE2 and enters the host cell. The density of ACE2 has a connection with the severity of the disease in the tissue (Gurwitz, 2020). The virus might not be detected in the central nervous system in the majority of the COVID-19 people with neurological conditions. However, SARS-CoV-2 has been seen at low levels in the brains of those who have died from COVID-19; however, these results are yet to be confirmed (Pezzini and Padovani, 2020). Loss of smell results from the infection of the supportive cells of the olfactive epithelial tissue, which later causes damage to the olfactive neurons (Meunier et al., 2021). There is no specific, effective treatment or cure for coronavirus disease 2019 (COVID-19). Therefore, the cornerstone for the management of COVID-19 is treating the symptoms, immediate oxygen support, fluid medical care, and medications or devices to support other affected organs (Siemieniuk et al., 2020; Liu et al., 2020). The development and production of drugs for curing permanently might take many months or years; the current situation indicates the necessity for locating various rapid treatments, vaccinations, and antiviral medicines as primary treatment options for combating or avoiding virus diseases from infection. Several studies on medicinal plant extracts and pure molecules were taken against coronavirus strains. In India, most people are consuming Kabasura Kudineer to prevent themselves from COVID-19 infection.

Kabasura Kudineer (KSK) or choornam possesses potent anti-inflammatory drug, analgesic, anti-viral, anti-bacterial, anti-fungal, inhibitor, hepato-protective, anti-pyretic, anti-asthmatic and immunomodulatory properties for managing several types of infections. A study unveiled that KSK has medicinal properties to aid the reduction of swelling inside the air passages. KSK includes 15 herbal ingredients such as Ginger (*Zingiber officinale*), Long pepper (*Piper longum*), Clove (*Syzygium aromaticum*), Dusparsha (*Tragia involucrata*), Kokilaksha (*Hygrophila auriculata*), Haritaki (*Terminalia chebula*), Malabar nut (*Justicia adhatoda*), Ajwain (*Coleus aromaticus*), Kusta (*Costus speciosus*), Guduchi (*Tinospora cordifolia*), Bharangi (*Clerodendrum serratum*), Akarakarabha (*Anacyclus pyrethrum*), Kalamegha (*Andrographis paniculata*),

Raja pata (*Sida acuta*), Musta (*Cyperus rotundus*) (Mekala and Murthy, 2020). In this study, we attempted to identify the activity of the phytoconstituents present in KSK, which were retrieved from the literature and Dr. Duke's Phytochemical and Ethnobotanical phytochemical database (<https://phytochem.nal.usda.gov/>), to compare them with synthetic drugs having immunomodulatory activity. KSK formulation is safe when taken along with homeopathic medicine (Figure 1).

Figure 1 The overall workflow of the study (see online version for colours)



2 Materials and methodology

2.1 Target gene identification and selection

Genes involved in the signalling pathways of COVID-19 were retrieved from the KEGG pathway database (Kanehisa and Goto, 2000; <https://www.genome.jp/kegg/pathway.html>). KEGG database is a high resource for finding high-level functions and utilities of biological systems. It has different windows like main entry point, data-oriented entry points, organism-specific entry points and analysis tools. *RIG-I-like receptor signalling pathway*, *IFN signalling pathway/Toll-like receptor signalling pathway*, and *SARS-CoV2S with Ang II-AT1R-NOX2 signalling pathway* (Coronavirus disease pathway) were the major signalling pathways involved in the pathogenesis of COVID-19 infection.

2.2 Network and functional analysis of selected genes

The protein-protein interaction network of the selected genes was constructed and analysed using the STRING database (Szklarczyk et al., 2021). The PPI network was visualised in *Cytoscape* software version 3.6.0 (Otasek et al., 2019) to detect the molecular mechanisms of key signalling pathways and cellular activities. *CytoHubba* (Chin et al., 2014), a Cytoscape plug-in, was used to find the essential genes and central elements in the network. The top 10 genes IRF3, IRF7, TNF, STAT1, IFNAR1, JAK1, IFNB1, STAT2, RELA and IL1B were selected by MCC method. MCC method revealed the degree of connectivity based on the most interconnected nodes. Among them, gene IRF3, the ultimate top gene, was the target for other processes. The three-dimensional protein structure of the chosen top hub gene was retrieved from Protein Data Bank (PDB) database (Sussman et al., 1998) based on its high resolution (1.80 Å) for further analyses. Additionally, the following studies were done using the miRNet database (Chang et al., 2020): KEGG pathways, Reactome analysis, Gene Ontology analyses (Biological Process, Molecular Function and Cellular Component) miRNAs of the target gene were also identified (Doria-Belenguer et al., 2023).

2.3 *Physical and chemical properties*

The target gene was computed based on the parameters such as molecular weight (calculated by the addition of the average isotopic mass of amino acids in the provided protein, and the average isotopic mass of one water molecule), theoretical pI (calculated using pKa values of amino acids), amino acid composition, atomic composition, extinction coefficient (describes how much light a protein absorbs at a specific wavelength), estimated half-life, instability index (provides stability of protein), aliphatic index (represents relative volume occupied by the amino acids such as alanine, valine, isoleucine and leucine have aliphatic side chain in their structure) and grand average of hydropathicity (calculated by adding the hydropath values of each amino acid residues and dividing by the number of residues in the sequence or length of the sequences) via ProtParam tool (Gasteiger et al., 2005; <http://www.expasy.org/tools/protparam.html>). In addition, the active site of the given gene was also predicted using the CastP server (Binkowski et al., 2003; <http://sts.bio.uic.edu/castp/>), which is an online database for functional site prediction. Structures of the proteins and their precalculated pocket measurements were retrieved by PDB ID from the CastP server. The measurements of all pocket sizes are based on the surface area and volume of the particular region of the protein. The following table includes the surface area and volume of proteins.

2.4 *Selection of active compounds*

Kabasura Kudineer is a well-known Siddha medicine that consists of 15 herbal ingredients, each of them having unique characteristic features of their own. Active compounds present in the herbal plants of Kabasura Kudineer were identified and retrieved from the literature and Dr. Duke's Phytochemical and Ethnobotanical Database (Lans and Asseldonk, 2020; <https://phytochem.nal.usda.gov/phytochem/search>). The structures of the selected compounds were downloaded from the PubChem database (.sdf) (Kim et al., 2016; <https://pubchem.ncbi.nlm.nih.gov/>).

2.5 *Virtual screening of ligands against the target gene*

PyRx (Dallakyan and Olson, 2015) is free open source virtual screening software used mainly in computational drug discovery domain to screen libraries of compounds against potential drug targets. This tool combines several tools like AutoDock and AutoDockVina for docking purposes. It is possible to select and delete the active sites and chains if needed to get appropriate results. Vina calculations were finally done, and the results will show the binding affinity of the protein-ligand complex displayed on the PyRx screen. The more the negative value of the binding relationship, the better the orientation of ligands in the binding site.

2.6 *Visualisation of protein-ligand interaction*

After virtual screening is done, the next step is to visualise the interaction of the protein and ligand complexes, where the output files of the results were exported to the PyMOL

tool (Yuan et al., 2017). These files were imported in PyMOL, and the protein-ligand complexes were saved as a.pdb file for further analysis (to view the interaction of protein and ligands). The exported PDB file of the protein-ligand file was loaded in LigPlot+ (Laskowski and Swindells, 2011) to view the 2D interaction of the imported PDB file of the protein-ligand complex for a better understanding of the interaction and the view of the bonding between the protein active site residues (Alonso et al., 2020) and the ligand.

2.7 Pharmacokinetic properties prediction

The Lipinski rule of five assists in discerning non-drug-like and drug-like candidates. It promises a high chance of success or failure due to drug likeness for molecules abiding by two or more of the complying rules. The following criteria support Lipinski's rule of five and determine whether the selected compounds obey five and have proper ADMET properties:

- i less than five hydrogen bond donors
- ii less than 10 hydrogen bond acceptors
- iii molecular mass less than 500 Da
- iv high lipophilicity (expressed as LogP, not over 5).

Lipinski rule and ADMET properties of the active compounds were examined using pkCSM (Pires et al., 2015; <http://biosig.unimelb.edu.au/pkcsm/>) and Molinspiration (Ertl et al., 2000; <https://www.molinspiration.com/>) online tools.

3 Results

3.1 Selection of target genes

The genes involved in the signalling pathways of COVID-19 were collected from the KEGG pathway database. Forty-six genes were involved in the mentioned signalling pathways and further taken for the network and functional analysis for identifying the top hub genes (Lingamgunta et al., 2023) among the selected genes (Table 1).

3.2 Network and functional analysis of selected genes

The selected genes were taken for constructing a protein-protein interaction network in the STRING database, and the constructed network has 45 nodes and 465 edges. Furthermore, the resultant network was visualised in Cytoscape software to detect the pathways' molecular mechanisms mentioned. cytoHubba, a plug-in tool of Cytoscape, was used to identify the top 10 hub genes of the network according to the degree of connectivity of the genes and where node scores were calculated. Finally, the top 10 nodes were ranked by the MCC method (Figure 2). IRF3, IRF7, TNF, STAT1, IFNAR1, JAK1, IFNB1, STAT2, RELA and IL1B were the top 10 hub genes. IRF3 was ranked 1,

taken as the leading hub gene by the ranking method, and selected as the target gene for further analysis. Using the miRNet online tool, Gene Ontology functions (Functional Enrichment analyses) of the target gene (IRF3) were examined, which describes KEGG pathways, Reactome, GO Biological Pathways, Molecular Functions and Cellular Components (Figure 3) (Table 2). The cutoff criteria for $pval < 0.05$ were taken to analyse the significant pathways and GO terms. IRF3 was a gene highly enriched in Cytosolic DNA-sensing pathway, RIG-I-like receptor signalling pathway, Pertussis, and Epstein-Barr virus infection. Defence response to the virus, positive regulation of cytokine production, intracellular receptor-mediated signalling pathway, and positive regulation of defence response were highly significant biological processes of the IRF3 gene. Transcription cofactor, protein homodimerisation and protein binding transcription factor activities were the essential molecular functions of IRF3. The gene IRF3 was significantly found in the endosome (a cellular component of IRF3).

Table 1 List of genes selected from signalling pathways of COVID-19

RIG-I-like receptor signalling pathway	DDX58, MAVS, TRAF3, TANK, AZ12, TBKBP1, TBK1, IKKBE, IRF3, IFNA1, IFNA2, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17, IFNA21, IFNB1, IRF7
IFN signalling pathway/Toll-like receptor signalling pathway	IFNB1, IFNA1, IFNA2, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17, IFNA21, IFNAR1, IFNAR2, JAK1, TYK2, STAT1, STAT2, IRF9, IRF7, EIF2AK2
SARS-CoV2S to Ang II-AT1R-NOX2 signalling pathway	ACE2, AGTR1, CYBB, NFB1, RELA, TNF, IL6, IL12A, IL12B, MMP3, MMP1, CCL2, CXCL8, IL1B

Figure 2 (A) PPI network constructed for the listed genes and (B) top 10 hub genes (see online version for colours)

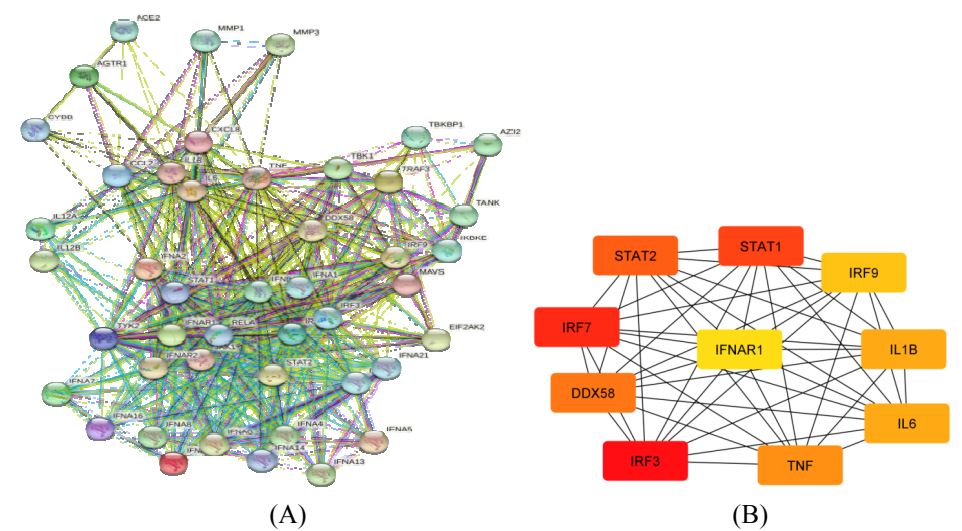


Figure 3 Functional enrichment analyses: pathway analysis (KEGG) and gene ontology analysis (BP, MF, and CC) (see online version for colours)

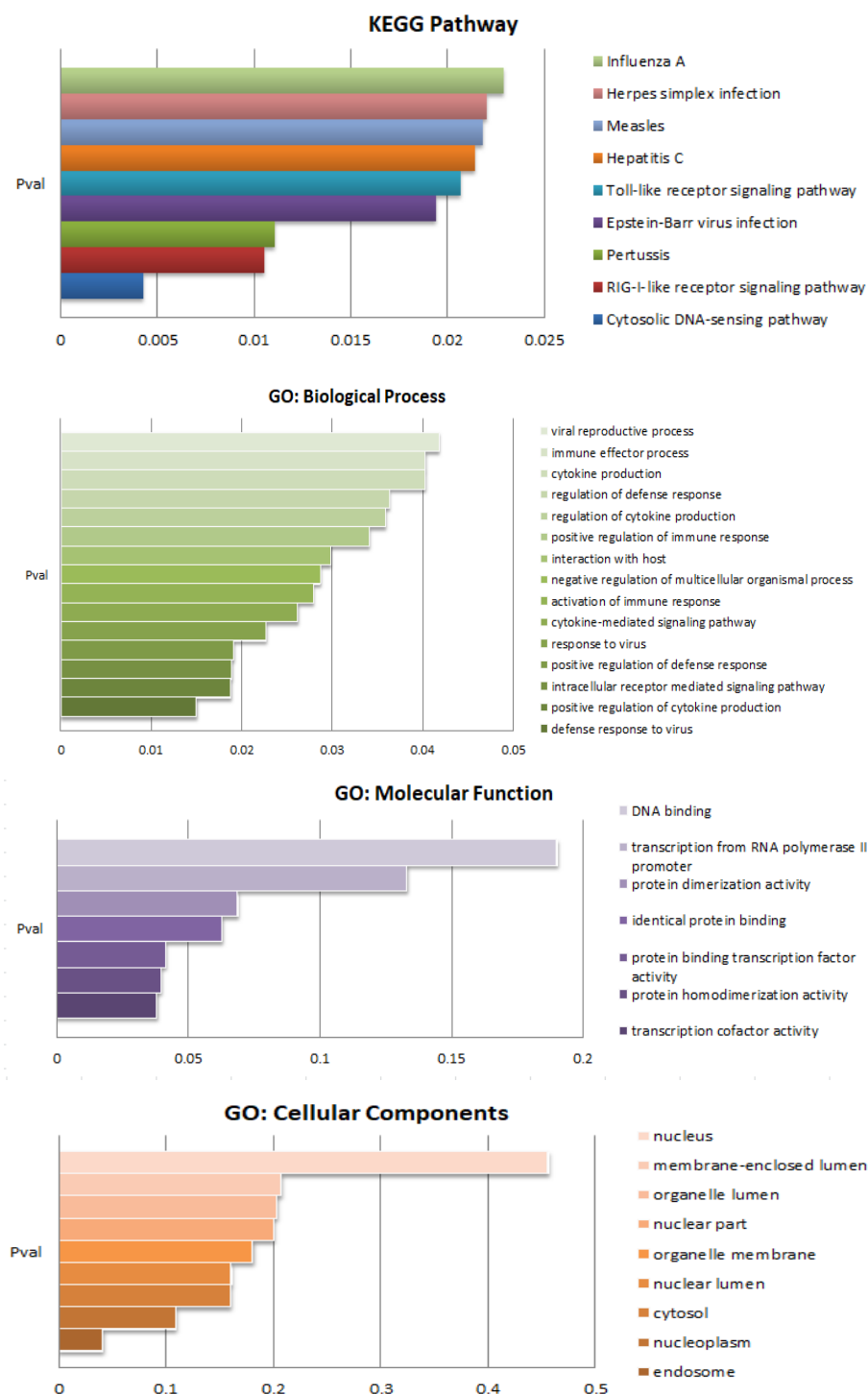


Table 2 Functional enrichment analyses (KEGG pathways, reactome, GO: biological process, molecular functions and cellular components) of IRF3 gene

<i>Pathway</i>	<i>Total</i>	<i>Expected</i>	<i>Hits</i>	<i>Pval</i>
<i>KEGG PATHWAY</i>				
Cytosolic DNA-sensing pathway	20	0.00427	1	0.00427
RIG-I-like receptor signalling pathway	49	0.0105	1	0.0105
Pertussis	52	0.0111	1	0.0111
Epstein-Barr virus infection	91	0.0194	1	0.0194
Toll-like receptor signalling pathway	97	0.0207	1	0.0207
Hepatitis C	100	0.0214	1	0.0214
Measles	102	0.0218	1	0.0218
Herpes simplex infection	103	0.022	1	0.022
Influenza A	107	0.0229	1	0.0229
<i>GO: BIOLOGICAL PROCESS</i>				
Defence response to virus	215	0.015	1	0.015
Positive regulation of cytokine production	268	0.0188	1	0.0188
Intracellular receptor-mediated signalling pathway	270	0.0189	1	0.0189
Positive regulation of defence response	273	0.0191	1	0.0191
Response to virus	325	0.0227	1	0.0227
Cytokine-mediated signalling pathway	374	0.0262	1	0.0262
Activation of immune response	399	0.0279	1	0.0279
Negative regulation of the multicellular organismal process	410	0.0287	1	0.0287
Interaction with host	426	0.0298	1	0.0298
Positive regulation of immune response	487	0.0341	1	0.0341
Regulation of cytokine production	513	0.0359	1	0.0359
Regulation of defence response	519	0.0363	1	0.0363
Cytokine production	576	0.0403	1	0.0403
Immune effector process	576	0.0403	1	0.0403
Viral reproductive process	597	0.0418	1	0.0418
<i>GO: MOLECULAR FUNCTION</i>				
transcription cofactor activity	552	0.0381	1	0.0381
protein homodimerisation activity	573	0.0396	1	0.0396
protein binding transcription factor activity	600	0.0414	1	0.0414
identical protein binding	910	0.0628	1	0.0628
protein dimerisation activity	996	0.0688	1	0.0688
transcription from RNA polymerase II promoter	1930	0.133	1	0.133
DNA binding	2760	0.19	1	0.19

Table 2 Functional enrichment analyses (KEGG pathways, reactome, GO: biological process, molecular functions and cellular components) of IRF3 gene (continued)

<i>Pathway</i>	<i>Total</i>	<i>Expected</i>	<i>Hits</i>	<i>Pval</i>
<i>GO: CELLULAR COMPONENTS</i>				
Endosome	683	0.041	1	0.041
Nucleoplasm	1820	0.109	1	0.109
Cytosol	2660	0.16	1	0.16
Nuclear lumen	2690	0.161	1	0.161
Organelle membrane	3020	0.181	1	0.181
Nuclear part	3330	0.2	1	0.2
Organelle lumen	3380	0.203	1	0.203
Membrane-enclosed lumen	3440	0.207	1	0.207
Nucleus	7600	0.456	1	0.456

In addition, miRNAs targeting gene and gene transcription factors were also determined using the miRNet tool. For the hub gene IRF3, two Transcription Factors and 62 miRNAs were involved. This information has been given in the network diagram and tabular form (Figure 4 and Table 3). The target gene was searched in PDB to retrieve the three-dimensional crystal structure of the protein to perform molecular docking studies against the ligand molecules. The PDB ID 3A77 (protein structure of the target gene) was downloaded based on its high resolution. The active site residues of the protein structure were also detected using CastP online server. The active sites for the chain A have been mentioned here because a unique ligand has been attached to all four chains, hence only one chain is enough to be taken for further steps (Table 4).

Table 3 Transcription factors and MiRNAs associated with the IRF3 gene

<i>Gene name</i>	<i>IRF3; Degree = 64; Betweenness = 2016</i>
<i>Transcription factors</i>	<i>E2F1, IRF4</i>
miRNAs	hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-let-7g-5p, hsa-let-7i-5p, hsa-mir-10a-3p, hsa-mir-1226-3p, hsa-mir-1254, hsa-mir-1277-3p, hsa-mir-130b-5p, hsa-mir-1343-3p, hsa-mir-148a-3p, hsa-mir-148b-3p, hsa-mir-152-3p, hsa-mir-15a-3p, hsa-mir-185-3p, hsa-mir-1910-5p, hsa-mir-197-3p, hsa-mir-1976, hsa-mir-2110, hsa-mir-219a-5p, hsa-mir-221-5p, hsa-mir-22-3p, hsa-mir-2355-3p, hsa-mir-2355-5p, hsa-mir-296-5p, hsa-mir-3157-3p, hsa-mir-3179, hsa-mir-3180-3p, hsa-mir-3200-3p, hsa-mir-320e, hsa-mir-324-3p, hsa-mir-338-3p, hsa-mir-342-3p, hsa-mir-346, hsa-mir-34a-3p, hsa-mir-34a-5p, hsa-mir-34c-5p, hsa-mir-361-3p, hsa-mir-3657, hsa-mir-3677-5p, hsa-mir-3913-5p, hsa-mir-4286, hsa-mir-449a, hsa-mir-449b-5p, hsa-mir-4726-3p, hsa-mir-491-5p, hsa-mir-500b-5p, hsa-mir-504-5p, hsa-mir-522-5p, hsa-mir-532-3p, hsa-mir-5581-5p, hsa-mir-5690, hsa-mir-629-3p, hsa-mir-660-3p, hsa-mir-660-5p, hsa-mir-766-3p, hsa-mir-769-5p, hsa-mir-98-5p, hsa-mir-1-3p, hsa-mir-129-2-3p, hsa-mir-146a-5p

3.3 Physical and chemical properties

The physical and chemical properties of the protein were analysed using the ProtParam tool. The computed parameters for calculating the properties include molecular weight, amino acid composition, theoretical pI, instability index, extinction coefficient, aliphatic index, and grand average of hydropathicity (GRAVY) (Table 5).

Table 5 Physical and chemical properties of IRF3 gene analysed by ProtParam tool

Molecular weight		47219.28
Theoretical pI		5.17
Atomic composition	Carbon	2112
	Hydrogen	3242
	Nitrogen	582
	Oxygen	626
	Sulphur	13
	Extinction coefficient	89295
	Instability index	46.75
	Aliphatic index	76.44
GRAVY		-0.447

3.4 Ligands selection

The bioactive compounds (Table 6) present in the ingredients of the Kabasura Kudineer were retrieved from the literature and Dr. Duke's Phytochemical and Ethnobotanical database; the structures of those compounds were downloaded from the PubChem database. Synthetic drugs used for the treatment of COVID-19 were also retrieved from the DrugBank database (but were not experimentally US FDA drugs). Finally, the selected compounds were used for virtual screening against the target protein.

3.5 Virtual screening – docking and interaction analyses

The target protein and the selected ligands were loaded in the virtual screening tool and were docked for analysing the binding affinity and the interaction between the target protein and the ligand complexes. The biological interaction analyses for the ligands and the target protein identified the compound with the protein's highest binding affinity. More the negative value of the docking score (Pan et al., 2022) of the combination with the ligand and target protein means having better orientation and interaction. Among the 90 active compounds and 20 synthetic compounds taken, most ligands showed good to moderate binding affinity with the target protein. It is found that the active compounds Costunolide and Lupeol (Figure 5(a)) and the synthetic drugs Vazegepant and Methylprednisolone (Figure 5(b)) showed good to moderate binding affinity with the target protein. From the interaction analyses table, the amino acids Arg 338, Arg 227, Glu 310, Val 319, and especially Arg 341 played a central role in hydrogen bonding in most active compounds and synthetic drugs.

Figure 5(a) Molecular docking studies of active compounds (Costunolide and Lupeol) against IRF3 gene (protein-ligand complex and binding pocket) (see online version for colours)

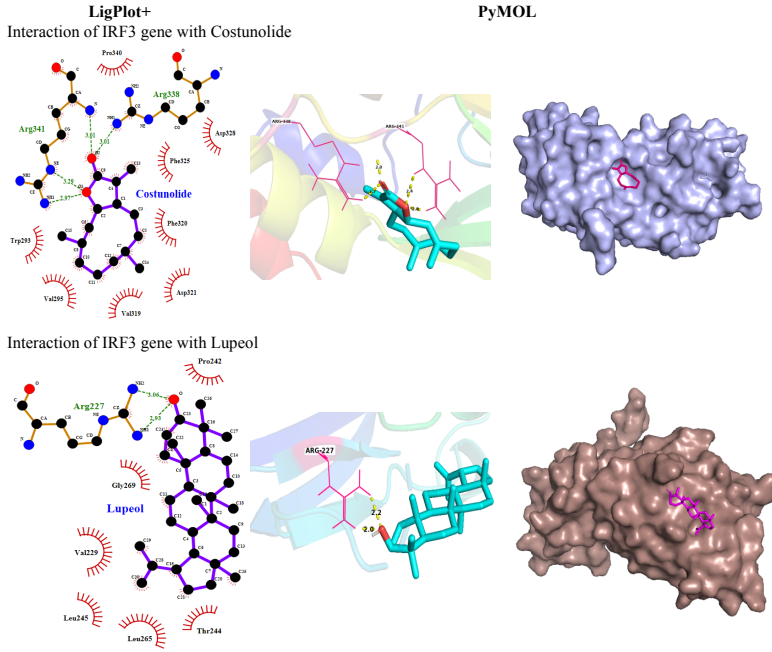


Figure 5(b) Molecular docking studies of synthetic drugs (Vazegepant and Methylprednisolone) against IRF3 gene (protein-ligand complex and binding pocket) (see online version for colours)

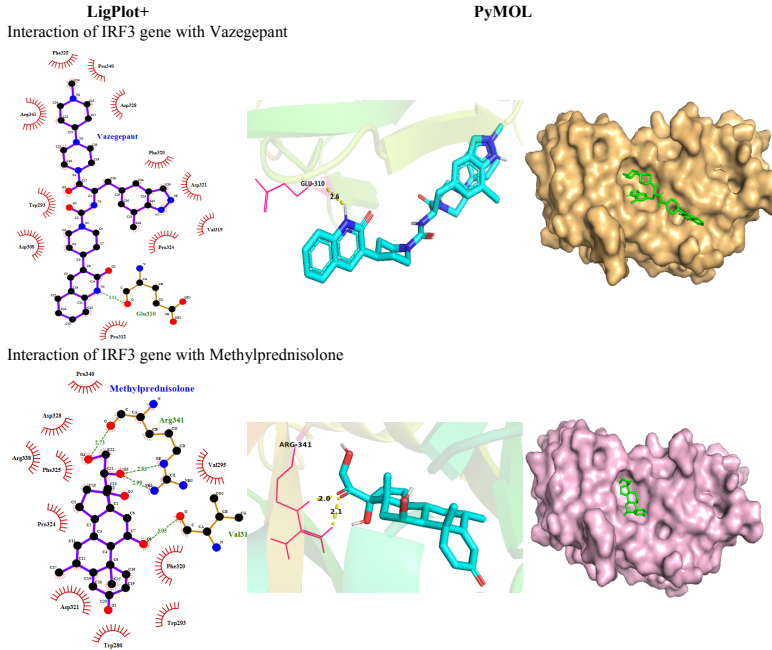


Table 6 Active compounds and synthetic drugs have a higher binding affinity to the target protein; interaction analyses and amino acid residues have roles in forming bonds with the protein (see online version for colours)

<i>Compound ID</i>	<i>Compound name</i>	<i>Binding affinity score</i>	<i>Plant name</i>	<i>Interactions</i>
<i>Active compounds</i>				
5281437	Costunolide	−9.2	<i>Costus speciosus</i>	No of HBonds: 4 Arg 341 N...O2 (3.01) NE...O1 (3.29) NH1...O1 (2.97) Arg 338 NH1...O2 (3.01)
259846	Lupeol	−9	<i>Costus speciosus</i> <i>Hygrophila auriculata</i>	No of HBonds: 2 Arg 227 NH1...O (2.93) NH2...O (3.06)
24721165	Isocolumbin	−8.8	<i>Tinospora cordifolia</i>	No of HBonds: 0 –
72326	Betulin	−8.6	<i>Hygrophila auriculata</i>	No of HBonds: 0 –
<i>Synthetic drugs</i>				
53472683	Vazegepant	−8.7	–	No of HBonds: 1 Glu 310 O...N3 (3.02)
6741	Methylprednisolone	−8	–	No of HBonds: 4 Arg 341 O...O4 (2.73) NE...O3 (2.93) NH1...O3 (2.99) Val 319 O...O5 (3.05)

3.6 Visualisation of interactions

The docking studies of all active compounds and synthetic drugs are compared to validate the docking and interaction studies of the active compounds with the target protein. The protein preparation, ligand preparation, minimisation, and docking were done in the PyRx Virtual Screening tool. PyMOL exported the structure output of PDB to 2D interaction of the protein-ligand complexes in the LigPlot + tool. The interaction between the protein-ligand complexes is visualised in three-dimensional and two-dimensional plots using PyMOL and LigPlot + tools (Figure 5). The three-dimensional

story of the protein-ligand complex shows the protein's binding pocket. The 2D plot determines the bonding between the active site residues of the protein and the ligand.

3.7 Molecular and pharmacokinetic property prediction

ADMET properties were determined to demonstrate the significant properties of those compounds; ADMET properties are the primary reason for the drug failure during the drug development phase. pkCSM and Molinspiration tools were used to examine the Lipinski rule of five and ADMET properties of the compounds to evaluate the drug likeliness and bioavailability. Here, the pharmacokinetic and molecular properties of the compounds are listed in Table 7.

Table 7 Molecular properties and pharmacokinetic properties (ADMET) of the best ligands

Property	Descriptor name	Predicted value			
		Costunolide	Lupeol	Vazegepant	Methylprednisolone
LRO5	Molecular weight	232.323	426.729	638.817	374.477
	LogP	3.5508	8.0248	3.45152	1.8036
	Rotatable Bonds	0	1	6	2
	HBondAcceptors	2	1	6	5
	HBondDonors	0	1	3	3
	Surface Area	103.043	192.398	274.824	159.706
	Molecular Weight	232.323	426.729	638.817	374.477
<i>Pharmacokinetic properties</i>					
Absorption	Water solubility	−3.764	−5.861	−3.109	−3.787
	Caco2 permeability	1.646	1.226	0.321	0.881
	Intestinal absorption (human)	97.18	95.782	69.86	73.366
	Skin Permeability	−2.423	−2.744	−2.735	−3.953
	P-glycoprotein substrate	No	No	Yes	No
	P-glycoprotein I inhibitor	No	Yes	Yes	No
	P-glycoprotein II inhibitor	No	Yes	Yes	No
Distribution	VDss (human)	0.31	0	1.676	−0.144
	Fraction unbound (human)	0.378	0	0.13	0.33
	BBB permeability	0.512	0.726	−1.051	−0.512
	CNS permeability	−2.672	−1.714	−2.766	−2.634

Table 7 Molecular properties and pharmacokinetic properties (ADMET) of the best ligands (continued)

Property	Descriptor name	Predicted value			
		Costunolide	Lupeol	Vazegepant	Methylprednisolone
Metabolism	CYP2D6 substrate	No	No	No	No
	CYP3A4 substrate	No	Yes	Yes	Yes
	CYP1A2 inhibitor	No	No	No	No
	CYP2C19 inhibitor	No	No	No	No
	CYP2C9 inhibitor	No	No	No	No
	CYP2D6 inhibitor	No	No	No	No
	CYP3A4 inhibitor	No	No	Yes	No
Excretion	Total Clearance	1.334	0.153	0.203	0.712
	Renal OCT2 substrate	No	No	No	Yes
Toxicity	AMES toxicity	No	No	No	No
	Max. tolerated dose (human)	0.449	−0.502	0.392	−0.183
	hERG I inhibitor	No	No	No	No
	hERG II inhibitor	No	Yes	Yes	No
	Oral Rat Acute Toxicity (LD50)	1.889	2.563	2.757	2.143
	Oral Rat Chronic Toxicity	1.943	0.89	4.254	0.654
	Hepatotoxicity	No	No	Yes	No
	Skin Sensitisation	Yes	No	No	No
	<i>T.Pyriformis</i> toxicity	0.921	0.316	0.285	0.345
	Minnow toxicity	0.844	−1.696	2.788	1.934

One of the essential pharmacokinetic properties is the human oral absorption of the compounds. The ligand properties were identified for selected compounds based on Lipinski's rule of 5 and ADMET properties. Here, the violations are less than 2 in all the compounds. So, all the compounds are eligible for further process. When comparing the phytocompounds and the synthetic drug compounds, both the phytocompounds (Costunolide and Lupeol) had good oral absorption values (97.18% and 95.782%, respectively) than the synthetic drug compounds.

4 Discussion

The COVID-19 pandemic has led to high death rates over a year worldwide. SARS-CoV-2 is one of the strains of the novel respiratory coronavirus. Several studies have been conducted on COVID-19 to find a more effective drug that can reduce the mortality rate. Many medical field experts are currently working on treating this dead infection. Siddha, Ayurveda, traditional herbal treatments like concoctions and allopathic medicines are also suggested to prevent getting infected by the virus. Currently, variants of Coronavirus like Delta and Omicron are becoming deadlier than their previous forms. Hence, an immediate precaution must be implemented to cure this infection. Kabasura Kudineer, or choornam possesses potent anti-inflammatory, analgesic, anti-viral, anti-bacterial, anti-fungal, antioxidant, hepato-protective, antipyretic, anti-asthmatic and immunomodulatory property which reduces swelling in air passages. At the same time, its antibacterial and antipyretic properties ease fever. Kabasura Kudineer carries 15 herbal ingredients, each of its properties (Kiran et al., 2020). This proposed study attempted to identify the target gene based on its function and potential drug compounds. The identification and selection of genes involved in the critical signalling pathways of COVID-19 were retrieved from the KEGG Pathway database. Protein-protein interaction analysis reveals the top 10 hub genes, and among them, the IRF3 gene has been ranked as a complete hub gene and thus considered for further analyses.

The body's innate immune response plays a vital role in combating viral infections as they produce interferon-alpha as the first line of defence against infection. It is revealed from the transcriptome analyses that those patients exposed to COVID-19 disease developed increased cytokine and interferon (IFN) signatures in the lungs. In particular, the initial response of IFN towards patients seems to be higher from mild to moderate level, and it was decreased when in critical condition. A recent study has revealed that IFN-alpha can be considered a potential therapeutic option for treating COVID-19 and has immunoregulatory properties (Yang et al., 2021). A research study demonstrated that parts of transcription factors like IRF3, IRF5, and p65 play a crucial role in regulating Interferon mRNA production in SARS-CoV-2 infected lung epithelium cells (Yin et al., 2021). Especially, IRF3 plays a vital role in driving the transcriptional regulation of type I IFN-dependent immune response against DNA and RNA viruses and even in SARS-CoV-2 infection (Tanaka and Chen, 2012; Liu et al., 2015; Zhao et al., 2016).

Based on the results obtained, among the 90 active compounds of 15 plants, most of the phytoconstituents were found to have a better binding affinity to the target protein when compared to the synthetic drugs. Lupeol is the most studied phytocompounds that possess anticancer, anti-diabetic, anti-inflammatory, and anti-malarial properties and is a non-hormonal male contraceptive (Somensi et al., 2021). A study performed by Singh et al. detected the possible inhibitory activity of the compound in controlling the consequences of SARS-CoV-2 infection by targeting the host immune system and viral targets (Singh et al., 2022). The synthetic drugs Vazegepant and Methylprednisolone have been used to treat COVID-19. Methylprednisolone has an anti-inflammatory and immunomodulatory effect, a synthetic corticosteroid (Zhu et al., 2020). Vazegepant is likely found to have combating activity in acute respiratory distress syndrome (ARDS) and can also be used in COVID-19 patients. Many drugs have anti-inflammatory, anti-

viral, and immunomodulatory activity against COVID-19 infection. However, the natural phytoconstituents present in herbal plants help boost immunity, and so the immune system itself can defend the body against disease. Such compounds also have little side effects than synthetic chemical drugs very much.

5 Conclusion

The overall results showed that the active compounds had a better binding affinity to the target protein when compared to the chemical drugs. These active compounds have antiviral and anti-inflammatory properties and excellent human oral absorption values. So, the predicted results such as pathway analysis, network analysis, functional analysis, physical and chemical property prediction, docking and pharmacokinetic property analysis are satisfying the eligible criteria. Further experimental studies have to be done to validate the results even more. This study highlights the immunomodulatory effects of the target gene IRF3, which drives the regulation of Interferon on SARS-CoV-2 infection. The phytoconstituents can enhance the activity of these transcription factors to improve the host's immunity against infection because preventing the disease is way better than taking treatment after getting infected.

Contributions

All authors wrote, reviewed, and edited the manuscript, analysed the data, provided resources, were responsible for data curation, and reviewed drafts of the paper. All authors read and approved the final version of the manuscript.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of interest

All authors declare that they have no conflicts of interest.

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References

- Alonso, S., Santiago, G., Cea-Rama, I., Fernandez-Lopez, L., Coscolin, C., Modregger, J., Ressmann, A.K., Martinez-Martinez, M., Marrero, H., Bargiela, R., Pita, M., Gonzalez-Alfonso, J.L., Briand, M.L., Rojo, D., Barbas, C., Plou, F.J., Golyshin, P., Shahgaldian, P., SanzAparicio, J., Guallar, V. and Ferrer, M. (2020) 'Genetically engineered proteins with two active sites for enhanced biocatalysis and synergistic chemo-and biocatalysis', *Nat Catal*, Vol. 3, pp.319–328.
- Binkowski, T.A., Naghibzadeh, S. and Liang, J. (2003) 'CASTp: computed atlas of surface topography of proteins', *Nucleic Acids Research*, Vol. 31, No. 13, pp.3352–3355.
- Chang, L., Zhou, G., Soufan, O. and Xia, J. (2020) 'miRNet 2.0: network-based visual analytics for miRNA functional analysis and systems biology', *Nucleic Acids Research*, Vol. 48, No. W1, pp.W244–W251.
- Chin, C.H., Chen, S.H., Wu, H.H., Ho, C.W., Ko, M.T. and Lin, C.Y. (2014) 'cytoHubba: identifying hub objects and sub-networks from complex interactome', *BMC Systems Biology*, Vol. 8, No. 4, pp.1–7.
- Dallakyan, S. and Olson, A.J. (2015) 'Small-molecule library screening by docking with PyRx', *Chemical Biology*, Humana Press, New York, NY, pp.243–250.
- Doria-Belenguer, S., Xenos, A., Ceddia, G., Malod-Dognin, N. and Pržulj, N. (2023) 'A functional analysis of omic network embedding spaces reveals key altered functions in cancer', *Bioinformatics*, Vol. 39, No. 5, pp.1–10.
- Ertl, P., Rohde, B. and Selzer, P. (2000) 'Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties', *Journal of Medicinal Chemistry*, Vol. 43, No. 20, pp.3714–3717.
- Gasteiger, E., Hoogland, C., Gattiker, A., Wilkins, M.R., Appel, R.D. and Bairoch, A. (2005) 'Protein identification and analysis tools on the ExPASy Server', in Walker, J.M. (Ed.): *The Proteomics Protocols Handbook*, Springer Protocols Handbooks, Humana Press.
- Gurwitz, D. (2020) 'Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics', *Drug Development Research*, Vol. 81, No. 5, pp.537–540.
- Harrison, A.G., Lin, T. and Wang, P. (2020) 'Mechanisms of SARS-coV-2 transmission and pathogenesis', *Trends in Immunology*, Vol. 41, No. 12, pp.1100–1115.
- Kanehisa, M. and Goto, S. (2000) 'KEGG: Kyoto encyclopedia of genes and genomes', *Nucleic Acids Research*, Vol. 28, No. 1, pp.27–30.
- Kim, S., Thiessen, P.A., Bolton, E.E., Chen, J., Fu, G., Gindulyte, A. and Bryant, S.H. (2016) 'PubChem substance and compound databases', *Nucleic Acids Research*, Vol. 44, No. D1, pp.D1202–D1213.
- Kiran, G., Karthik, L., Devi, M.S., Sathiyarajeswaran, P., Kanakavalli, K., Kumar, K.M. and Kumar, D.R. (2020) 'In silico computational screening of Kabasura Kudineer-official siddha formulation and JACOM against SARS-CoV-2 spike protein', *Journal of Ayurveda and Integrative Medicine*, Vol. 13, No. 1, pp.1–8.
- Lans, C. and Asseldonk, T.V. (2020) 'Dr. Duke's phytochemical and ethnobotanical databases, a cornerstone in the validation of ethnoveterinary medicinal plants, as demonstrated by data on pets in British Columbia', *Medicinal and Aromatic Plants of North America*, Springer, Cham, pp.219–246.
- Laskowski, R.A. and Swindells, M.B. (2011) 'LigPlot+: multiple ligand–protein interaction diagrams for drug discovery', *Journal of Chemical Information and Modeling*, Vol. 51, No. 10, pp.2778–2786.
- Lingamgunta, L.K., Aloor, B.P., Dasari, S., Ramakrishnan, R., Botlagunta, M., Madikonda, A.K., Gopal, S. and Sade, A. (2023) 'Identification of prognostic hub genes and therapeutic targets for selenium deficiency in chicks model through transcriptome profiling', *Scientific Reports*, Vol. 13, No. 8695, pp.1–16.

- Liu, K., Fang, Y.Y., Deng, Y., Liu, W., Wang, M.F., Ma, J.P., Xiao, W., Wang, Y.N., Zhong, M.H., Li, C.H., Li, G.C. and Liu, H.G. (2020) 'Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei province', *Chinese Medical Journal*, Vol. 133, No. 9, pp.1025–1031.
- Liu, S., Cai, X., Wu, J., Cong, Q., Chen, X., Li, T., Du, F., Ren, J., Wu, Y.T., Grishin, N.V. and Chen, Z.J. (2015) 'Phosphorylation of innate immune adaptor proteins MAVS, STING, and TRIF induces IRF3 activation', *Science*, Vol. 347, No. 6227, pp.aaa2630-1–aaa2630-14.
- Malone, B., Urakova, N., Snijder, E.J. and Campbell, E.A. (2022) 'Structures and functions of coronavirus replication–transcription complexes and their relevance for SARS-CoV-2 drug design', *Nature Reviews Molecular Cell Biology*, Vol. 23, No. 1, pp.21–39.
- Mekala, P. and Murthy, T.G. (2020) 'Phytochemical screening and pharmacological update on Kabasura Kudineer Choornam and Nilavembu Kudineer Choornam', *J. Pharmacognosy Phytochemistry*, Vol. 9, No. 3, pp.1031–1036.
- Meunier, N., Briand L., Jacquin-Piques, A., Brondel, L. and Pénicaud, L. (2021) 'COVID 19-induced smell and taste impairments: putative impact on physiology', *Frontiers in Physiology*, Vol. 11, p.1882.
- Otasek, D., Morris, J.H., Bouças, J., Pico, A.R. and Demchak, B. (2019) 'Cytoscape automation: empowering workflow-based network analysis', *Genome Biology*, Vol. 20, No. 1, pp.1–15.
- Pan, X., Wang, H., Zhang, Y., Wang, X., Li, C., Ji, C. and Zhang, J.Z.H. (2022) 'AA-score: A new scoring function based on amino acid-specific interaction for molecular docking', *Journal of Chemical Information and Modeling*, Vol. 62, No. 10, pp.2499–2509.
- Pezzini, A. and Padovani, A. (2020) 'Lifting the mask on neurological manifestations of COVID-19', *Nature Reviews Neurology*, Vol. 16, No. 11, pp.636–644.
- Pires, D.E., Blundell, T.L. and Ascher, D.B. (2015) 'pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures', *Journal of Medicinal Chemistry*, Vol. 58, No. 9, pp.4066–4072.
- Siemieniuk, R.A., Bartoszko, J.J., Zeraatkar, D., IzCoVich, A., Kum, E., Qasim, A., Martinez, J.P.D., Izcovich, A., Lamontagne, F., Han, M.A., Agarwal, A., Agoritsas, T., Azab, M., Bravo, G., Chu, D.K., Couban, R., Devji, T., Escamilla, Z., Foroutan, F., Gao, Y., Ge, L., Ghadimi, M., Heels-Ansdell, D., Honarmand, K., Hou, L., Ibrahim, Q., Khamis, A., Lam, B., Mansilla, C., Loeb, M., Miroshnychenko, A., Marcucci, M., McLeod, S.L., Motaghi, S., Murthy, S., Mustafa, R.A., Pardo-Hernandez, H., Rada, G., Rizwan, Y., Saadat, P., Switzer, C., Thabane, L., Tomlinson, G., Vandvik, P.O., Vernooij, R.W., Viteri-García, A., Wang, Y., Yao, L., Zhao, Y., Guyatt, G.H. and Brignardello-Petersen, R. (2020) 'Drug treatments for CoVid-19: living systematic review and network meta-analysis', *BMJ*, Vol. 370, No. m2980, pp.1–12.
- Singh, P., Chauhan, S.S., Pandit, S., Sinha, M., Gupta, S., Gupta, A. and Parthasarathi, R. (2022) 'The dual role of phytochemicals on SARS-CoV-2 inhibition by targeting host and viral proteins', *Journal of Traditional and Complementary Medicine*, Vol. 12, No. 1, pp.90–99.
- Somensi, L.B., Costa, P., Boeing, T., Bolda, M.L.N., de Gregório, E., E Silva, A.T.M., Longo, B., Locatelli, C., de Souza, P., Magalhães, C.G., Pains, D.L. and da Silva, L.M. (2021) 'Lupeol stearate accelerates healing and prevents recurrence of gastric ulcer in rodents', *Evid Based Complement Alternat Med.*, Vol. 2022, No. 6134128, pp.1–10.
- Sussman, J.L., Lin, D., Jiang, J., Manning, N.O., Prilusky, J., Ritter, O. and Abola, E.E. (1998) 'Protein data bank (PDB): database of three-dimensional structural information of biological macromolecules', *Acta Crystallographica Section D: Biological Crystallography*, Vol. 54, No. 6, pp.1078–1084.
- Swed, S., Alibrahim, H., Sawaf, B. and Hussein Al-Janabi, M.A. (2022) 'COVID-19 and random prescription of antibiotics in Syria', *Annals of Medicine and Surgery*, Vol. 73, No. 103181, pp.1–2.

- Szklarczyk, D., Gable, A.L., Nastou, K.C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N.T., Legeay, M., Fang, T., Bork, P., Jensen, L.J. and von Mering, C. (2021) 'The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets', *Nucleic Acids Research*, Vol. 49, No. D1, pp.D605–D612.
- Tanaka, Y. and Chen, Z.J. (2012) 'STING specifies IRF3 phosphorylation by TBK1 in the cytosolic DNA signaling pathway', *Science Signaling*, Vol. 5, No. 214, pp.ra20–ra20.
- Verdecchia, P., Cavallini, C., Spanevello, A. and Angeli, F. (2020) 'The pivotal link between ACE2 deficiency and SARS-CoV-2 infection', *European Journal of Internal Medicine*, Vol. 76, pp.14–20.
- Yang, L., Wang, J., Hui, P., Yarovinsky, T.O., Badeti, S., Pham, K. and Liu, C. (2021) 'Potential role of IFN- α in COVID-19 patients and its underlying treatment options', *Applied Microbiology and Biotechnology*, Vol. 105, No. 10, pp.4005–4015.
- Yin, X., Riva, L., Pu, Y., Martin-Sancho, L., Kanamune, J., Yamamoto, Y., Sakai, K., Gotoh, S., Miorin, L., De Jesus, P.D., Yang, C.C., Herbert, K.M., Yoh, S., Hultquist, J.F., García-Sastre, A. and Chanda, S.K. (2021) 'MDA5 governs the innate immune response to SARS-CoV-2 in lung epithelial cells', *Cell Reports*, Vol. 34, No. 2, pp.1–12.
- Yousefi, B. and Eslami, M. (2022) 'Genetic and structure of novel coronavirus COVID-19 and molecular mechanisms in the pathogenicity of coronaviruses', *Reviews in Medical Microbiology*, Vol. 33, No. 1, pp.e180–e188.
- Yuan, S., Chan, H.S. and Hu, Z. (2017) 'Using PyMOL as a platform for computational drug design', *Wiley Interdisciplinary Reviews: Computational Molecular Science*, Vol. 7, No. 2, p.e1298.
- Zhao, B., Shu, C., Gao, X., Sankaran, B., Du, F., Shelton, C.L., Herr, A.B., Ji, J.Y. and Li, P. (2016) 'Structural basis for concerted recruitment and activation of IRF-3 by innate immune adaptor proteins', *Proceedings of the National Academy of Sciences*, Vol. 113, No. 24, pp.E3403–12.
- Zhu, L., Xu, X., Ma, K., Yang, J., Guan, H., Chen, S., Chen, Z. and Chen, G. (2020) 'Successful ReCoVery of COVID-19 pneumonia in a renal transplant recipient with long-term immunosuppression', *American Journal of Transplantation*, Vol. 20, No. 7, pp.1859–1863.