Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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In silico screening of potential anti−COVID-19 bioactive natural constituents from food sources by molecular docking

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ABSTRACT

Objectives: The aim of this study was to seek potential natural compounds that can resist COVID-19 using computer virtual screening technology through molecular docking of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) 3CL hydrolytic enzyme ($3CL^{pro}$) and angiotensin-converting enzyme 2 (ACE2).
Methods: Molecular docking was achieved by using the Autodock Vina software. The natural phytocompounds acting on $3CL^{pro}$ and ACE2 were then selected from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform. This was followed by speculation on the mechanism of action of phytocompounds.
Results: Six potential natural anti−COVID-19 phytocompounds were selected and were evaluated for absorption, distribution, metabolism and excretion (ADME) and Lipinski rules. The content of the six phytocompounds in various fruits and vegetables was determined via a literature search. Red wine, Chinese hawthorn, and blackberry were recommended as supplements because they contained antiviral phytocompounds.
Conclusion: Red wine, Chinese hawthorn, and blackberry show promise for resisting COVID-19 and are thus recommended as supplements to prevent the infection of COVID-19 during its outbreak period.

Introduction

The coronavirus (CoV) is an infectious disease caused by a newly discovered coronavirus, characterized by rapid and extensive spread, strong infectivity, and general susceptibility of the population. Currently, there is no specific drug to treat or cure it. The new coronavirus was officially designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; formerly known as 2019-ncov) by the International Commission on the Classification of Viruses on February 11, 2020. On the same day, the World Health Organization named the disease caused by this virus as COVID-19. Coronaviruses are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East respiratory syndrome (MERS-CoV) and severe acute respiratory syndrome (SARS-CoV). At present, the homology between SARS-CoV-2 and bat Sars-like coronavirus (bat-sl-covzc45) is >85%. The s-protein expressed by SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) in the human body, infecting cells, invading the body, and causing diseases [1]. The human coronavirus 229E (*HCoV-229E*) replicase gene encodes two overlapping polyproteins pp1a and pp1ab that mediate all the functions required for viral replication and transcription. Expression of the C-proximal portion of pp1ab requires (−1) ribosomal frameshifting. The functional polypeptides are released from the polyproteins by extensive proteolytic processing, and that is primarily achieved by 3C-like proteinase (3CLpro) [2]. On January 26, 2020, a research team from Shanghai University of Science and Technology obtained a high-resolution crystal structure of 2019-nCoV coronavirus 3CL hydrolase (M^{pro}), which is considered an effective target of the COVID-19 virus [3]. These studies have brought hope to the search for effective drugs to prevent and control the COVID-19, and may help us to develop a more effective way to fight COVID-19.

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Scientists have been focusing on searching antiviral phytocompounds with low toxicity and high curative effect from natural plants in recent years. Natural active substances have the characteristics of novel structure, high activity, and few side effects. Natural compounds from plant origin currently used in medicine exhibit chemical diversity, demonstrating their importance in modern drug discovery efforts. There is a growing trend to explore plants for pharmacologically active compounds and nutraceutical supplements.

Molecular docking is a method of drug design based on the characteristics of the receptor and the way the receptor interacts with the drug molecule. As an emerging research method combining the physical and chemical principles with scientific calculation algorithms, molecular docking provides a feasible strategy for exploring the basis and mechanism of the phytocompounds [4]. This study took SARS-CoV-2 $3CL^{pro}$ and ACE2 as receptors, and molecular docking of the two was performed to select potential antiviral active ingredients for the development of effective and quick-acting chemical components that can resist COVID-19.

Materials and methods

Database and software

Data used in this study were downloaded from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP; http://tcmspw.com/tcmsp.php), Protein Databank (PDB; https://www.rcsb.org/), Swiss Target Prediction database (http://www.swisstargetprediction.ch/), STRING online database (https://string-db.org/), and biological information annotation database DAVID (https:// DAVID. Ncifcrf. Gov/summary. The JSP, Version 6.8). The software used included AutoDockTools1.5.6 software, AutoDockVina software (http://vina.scripps.edu/), biological information analysis tools Cytoscape v3.8.1, data analysis tools R 3.6.2, protein molecules, and visualization software PyMOL.

Molecular docking simulation

We selected 12 541 natural bioactive constituents of plants from the TCMSP database, and their three-dimensional (3D) structures were downloaded in SDF format to establish a virtual screening small molecule database. The interactions of two target proteins with phytocompounds and with currently recommended clinical chemical drugs were also compared. The 3D structures of the clinical drugs and ACE2 (PDB ID: 1R42) protein were downloaded from PubChem in SDF format and PDB data (https://www.rcsb.org/) in PDB format, respectively. SARS-CoV-2 is determined to be a high-resolution crystal structure (PDB ID: 6LU7) of SARS-CoV-2 $3CL^{pro}$ by a research group from Shanghai University of Science and Technology. PyMOL software was used for water removing, hydrogenation, and other operations, and high-throughput molecular docking was carried out by Autodock Vina and R.

Absorption, distribution, metabolism and excretion analysis and Lipinski's rule of five

Pharmacokinetic (PK) analysis of biological or pharmaceutically active compounds was conducted to select drug candidates [5]. Absorption, distribution, metabolism and excretion (ADME) screening criteria for ligands in this study included oral bioavailability (OB) >30% and drug-likeness (DL) >0.18. Values were obtained from the TCMSP database. Lipinski's rule of five is also called Pfizer's rule, which specifically includes relative molecular weight <500, ClogP <5, number of hydrogen bond donors <5, number of hydrogen bond receptors <10, number of keys ≤10, which are used to evaluate the DL and durability of a phytochemical or chemical compound. Compounds that conform to Lipinski's rule of five will have better PK properties and higher bioavailability in the metabolic process in vivo, and are therefore more likely to be made into oral drugs. In this study, the phytocompounds were further chosen from the small molecule database, which was an efficient way to find compounds with good PK properties and high bioavailability. Ligands of this particular study were analyzed using http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp based on Lipinski's rule of five.

Source seeking and molecular mechanism prediction by literature mining

According to the optimal binding energy (affinity) of phytocompounds with $3CL^{pro}$ and ACE2, potential anti−COVID-19 phytocompounds were identified through ADME analysis and Lipinski screening. Based on the goal of this research to find common and easily available food supplements that can help prevent COVID-19, literature mining was conducted using PubMed, Web of Science, and EBSCO to seek the food sources of these potential phytocompounds. The principle of literature mining is that as many compounds as possible are enriched in the same food source in the hope that it can prevent viruses to a greater extent, and the food is widely distributed and easily available.

In this study, SARS and viral pneumonia were used as references to search for potential targets of anti−COVID-19 phytocompounds. The potential targets of phytocompounds were predicted by the Swiss Target Prediction server. STRING database was employed to the analysis of the relationship between drugs and targets. Then, the visual analysis was carried out using Cytoscape software. Subsequently, the potential targets of the selected active components were submitted to the bioinformatics database DAVID 6.8 for functional annotation of gene ontology (GO) genes and enrichment analysis of KEGG and REACTOME pathways, to further investigate the functions of these targets and their role in the signaling pathways, thereby exploring and predicting the potential molecular mechanism of phytocompounds.

Results

Anti−COVID-19 phytocompounds

It is generally believed that the lower the stabilization energy of ligand binding to the receptor, the greater the possibility of action. To minimize the probability of false-positive results, the optimal binding energy of phytocompounds was compared with that of the currently recommended clinical chemical drugs in this study, and the binding energy in screening criteria was changed to ≤−5 kcal/mol (−20 kJ/mol). The partial results are shown in Table 1. Six potential anti−COVID-19 phytocompounds were chosen.

The molecular docking modes of the six potential anti−COVID-19 phytocompounds with SARS-CoV-2 $3CL^{pro}$ and ACE2 are shown in Figure 1.

Table 1
Binding energy (kJ/mol) of representative phytocompounds and clinically recommended chemical drugs with SARS-CoV-2 $3CL^{pro}$ and ACE2

Molecule	Formula	MW	$3CL^{pro}$	ACE2	DL	OB (%)	Lipinski
Puerarin	C21H20O10	432.38	−33.47	−38.07	0.69	24.03	Yes
Bicuculline	C20H17NO6	367.4	−26.78	−41.42	0.88	69.67	Yes
Luteolin	C15H10O6	286.24	−26.78	−36.82	0.25	36.16	Yes
Quercetin	C15H10O7	302.24	−26.36	−36.40	0.28	46.43	Yes
Isorhamnetin	C16H12O7	316.27	−25.95	−35.15	0.31	49.6	Yes
Irisolidone	C17H14O6	314.29	−25.53	−38.49	0.3	37.78	Yes
Lopinavir	C37H48N4O5	628.8	−22.59	−37.24			
Ritonavir	C37H48N6O5S2	720.94	−24.69	−36.40			
Remdesivir	C27H35N6O8P	602.58	−25.94	−36.40			
Arbidol	C22H25BrN2O3S	531.89	−28.03	−30.54			
Chloroquine	C18H26ClN3	319.87	−24.3	−27.20			
Ribavirin	C37H48N6O5S2	720.96	−25.52	−32.22			
Nitazoxanide	C12H9N3O5S	307.28	−23.85	−34.73			

ACE, angiotensin-converting enzyme; DL, drug-likeness; MW, molecular weight; OB, oral bioavailability; SARS-CoV-2 $3CL^{pro}$, severe acute respiratory syndrome coronavirus 3CL hydrolytic enzyme.

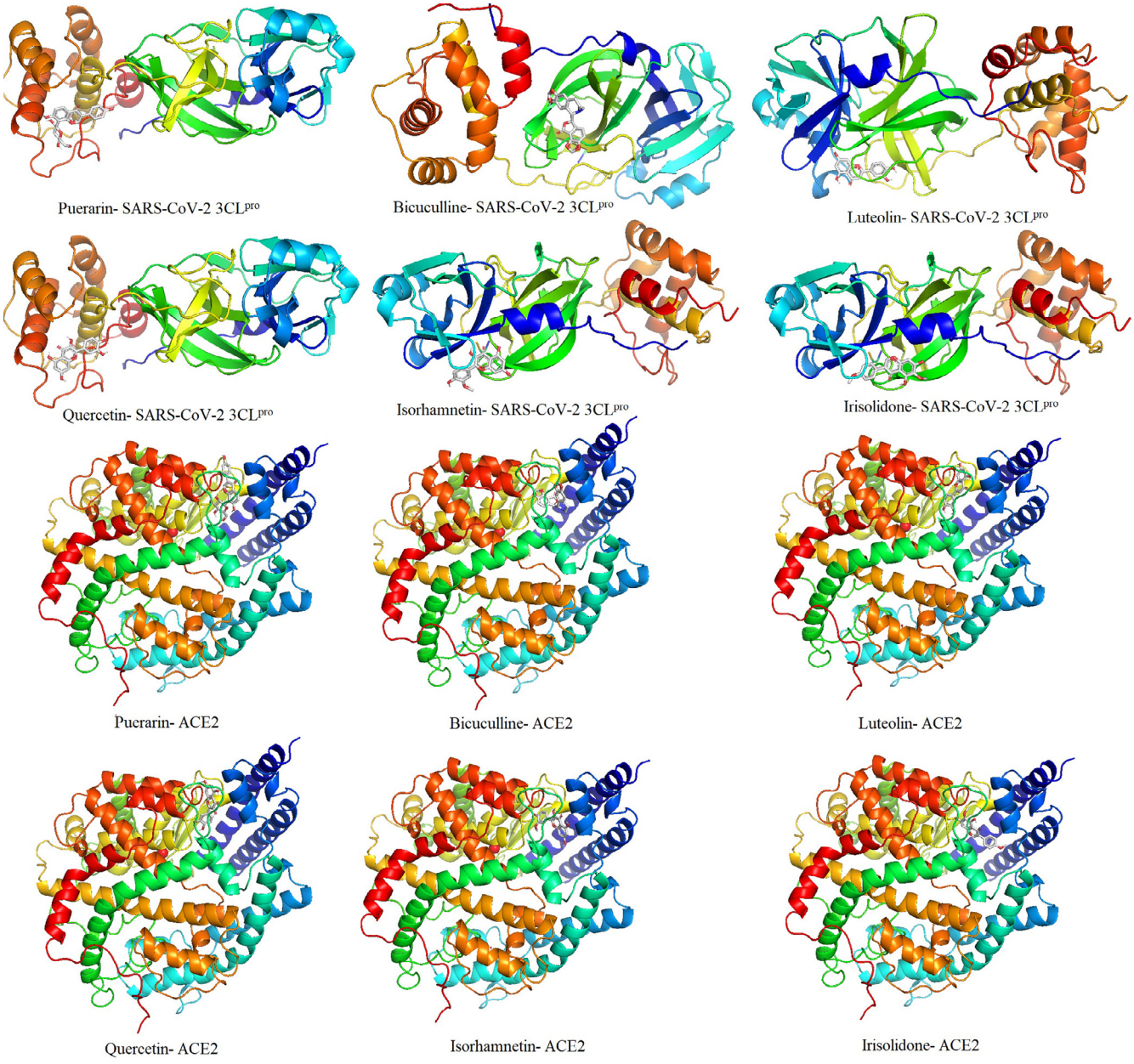

Fig. 1. Molecular docking patterns of 6 potential anti-COVID-19 phytocompounds with SARS-CoV-2 3CL^{pro} and ACE2. ACE, angiotensin-converting enzyme; SARS-CoV-2 3CL^{pro}, severe acute respiratory syndrome coronavirus 3CL hydrolytic enzyme.

According to the ADME screening criteria, puerarin was eliminated due to its relatively low OB. The remaining five plant compounds were included in further literature mining to enrich as many candidate compounds as possible in the same food source.

Bioactive natural constituents from food sources

A large-scale literature search was performed to seek the food sources of potential bioactive natural constituents. On the basis of the enrichment results of the preferred plant ingredients (Fig. 2), we excluded five uncommon foods and finally set the focus of the research on red wine, Chinese hawthorn, and blackberry enriched with three phytocompounds (quercetin, luteolin, and isorhamnetin).

Quercetin is a bioflavonoid widely present in red wine, grapefruit, onions, apples, and black tea, having antioxidant and anti-inflammatory activity. A lesser amount of it exists in leafy green vegetables and beans [6]. Red wine is shown to promote quercetin absorption and direct its metabolism toward isorhamnetin and tamarixetin [7]. Several studies have determined luteolin and isorhamnetin in red wine. Additionally, luteolin (0.11–3.99 mg/L), isorhamnetin (0–0.62 mg/L) and quercetin (0.04–2.65 mg/L) were detected in 17 kinds of commercial red wine made in China [8].

Quercetin and isorhamnetin are the most abundant flavonoids in plant-based foods [9]. Maja Mikulic-Petkovsek [10] detected two antiviral phytocompounds (quercetin and isorhamnetin) in 28 wild and cultivated berries (including Chinese hawthorn and blackberry) from around the world. Quercetin and isorhamnetin were found to be always present in all red-skinned species in this study. Luteolin has been identified in both Chinese hawthorn [11] and blackberry [12] in recent studies.

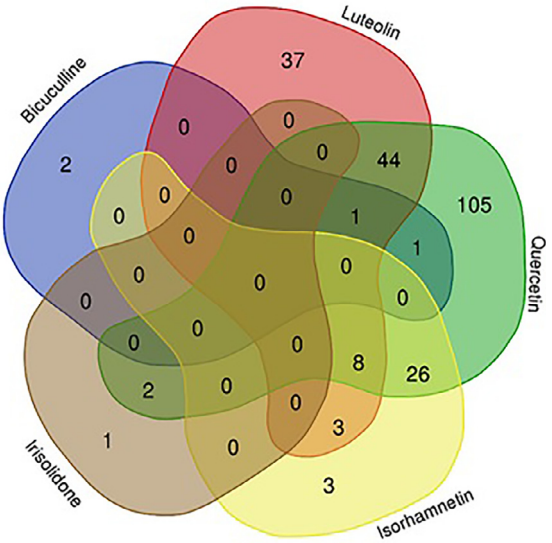

Fig. 2. Enrichment results of the preferred plant ingredients.

Red wine, Chinese hawthorn, and blackberry are enriched with three of the five anti–COVID-19 phytocompounds, and can be found in almost every corner of the world, so they are recommended as anti–COVID-19 food supplements.

Network pharmacologic analysis of quercetin, luteolin, and isorhamnetin

The potential targets of three bioactive natural constituents (quercetin, luteolin, and isorhamnetin) and the disease targets were input into the R platform for the identification of the intersection between the two kinds of targets. The Venn diagram in Figure 3 shows the overlap of the two kinds of targets.

Quercetin, luteolin, and isorhamnetin are very similar to disease targets as they are flavonoids. In addition to the two targets used in this study, there are 41 similar targets acting on both SARS and viral pneumonia. The topologic analysis of the protein interaction network between the 41 targets is shown in Figure 4.

To illustrate the mechanism underlying the effects of quercetin, luteolin, and isorhamnetin on COVID-19 more comprehensively and specifically, we performed GO enrichment analysis of the

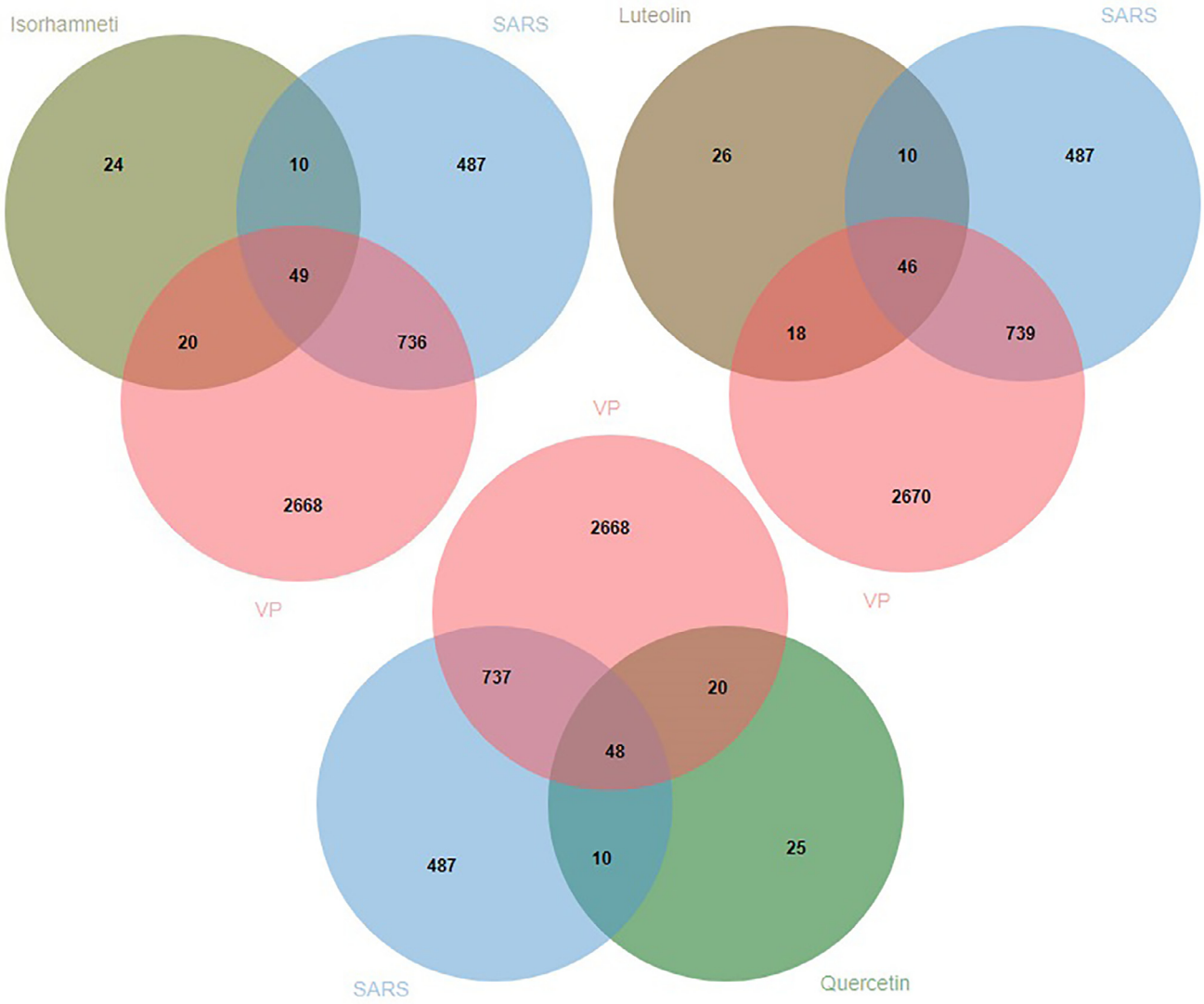

Fig. 3. Venn diagram of intersected targets. SARS, severe acute respiratory syndrome; VP,Viral Pneumonia.

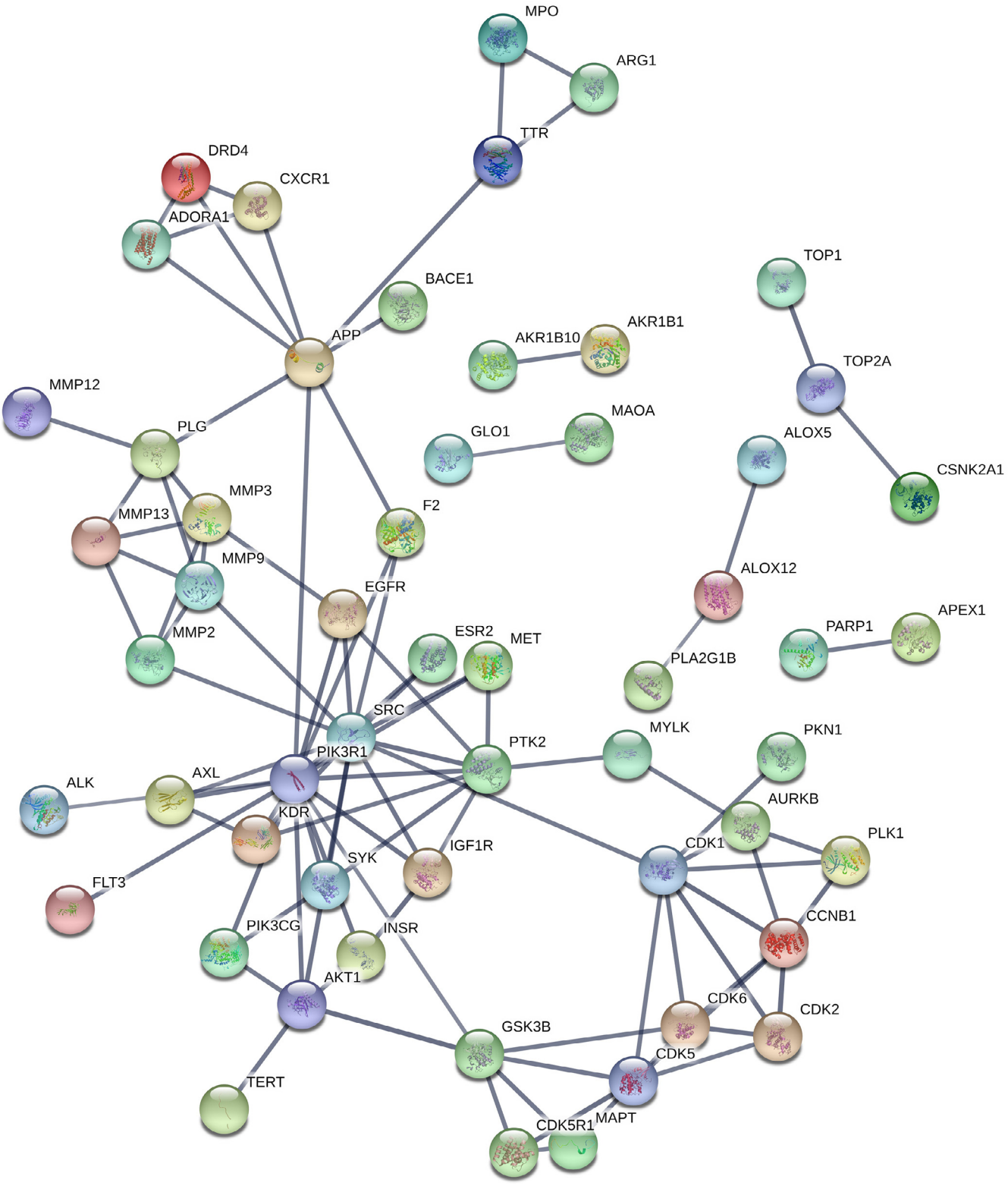

Fig. 4. Network of overlapping targets.

intersected targets in the ingredient-disease target network. We found that 1109 GO terms were significantly enriched in the biological process, 130 in the molecular function, and 115 in the cellular component. The smallest *P*-adjusted value was observed in response to oxygen-containing compounds, regulation of cell death, catalytic activity, and protein kinase activity.

The enrichment analysis of KEGG and REACTOME pathways was carried out to elucidate the critical pathways of the potential

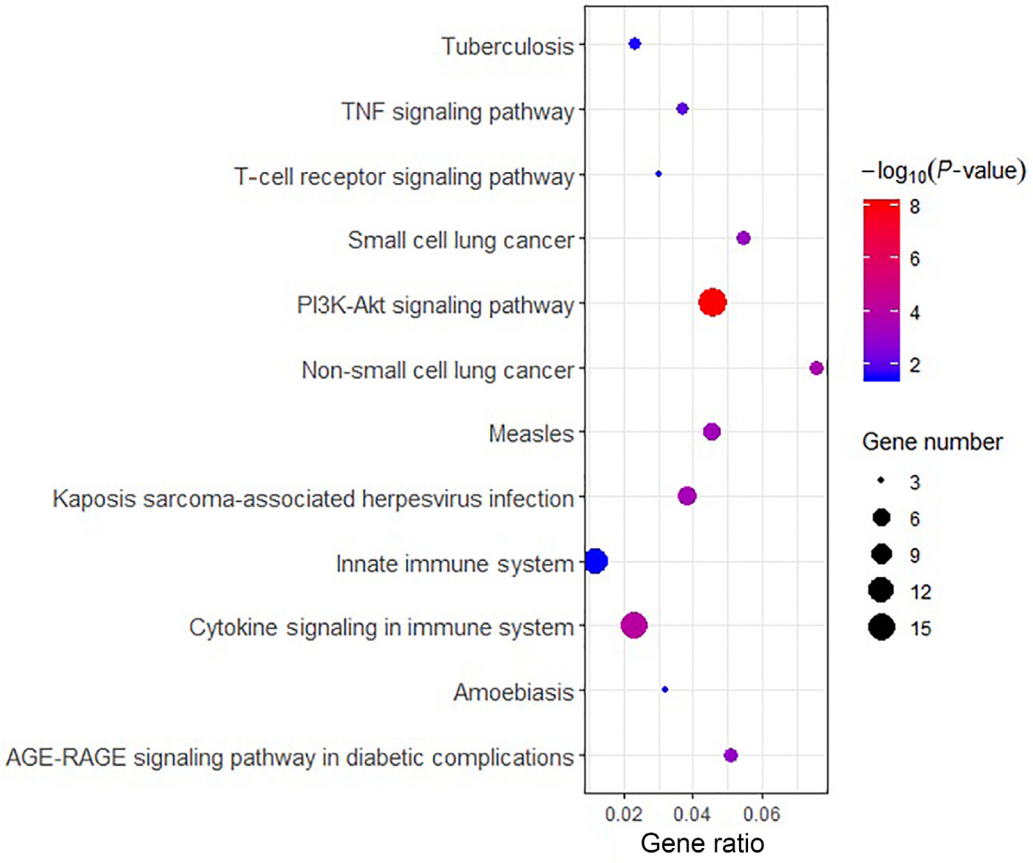

Fig. 5. Overlapping potential pathways selected. AGE-RAGE, advanced glycation end products-receptor for advanced glycation end products; TNF, tumor necrosis factor.

targets for quercetin and isorhamnetin in resisting COVID-19. It was observed that 130 pathways were significantly enriched in KEGG and 172 in REACTOME. After removing the duplicates, the results were compared with the super pathways of SARS and viral pneumonia. Twelve overlapping potential pathways were ultimately selected (Fig. 5).

Discussion

In this study, six phytocompounds from natural plants with low binding energy to receptors were first selected through molecular docking. Red wine, Chinese hawthorn, and blackberry are recommended as anti−COVID-19 supplements because they contain two or three active phytocompounds (ie, quercetin, luteolin, and isorhamnetin), as indicated by extensive literature search. Reports have suggested that flavonoids, as a large class of natural compounds, might be useful for the prevention of a number of diseases, partly due to their anti-inflammatory properties [13]. Quercetin, luteolin, and isorhamnetin were shown to have antioxidant and anti-inflammatory effects in previous studies [6,14,15]. The results of a recent study showed that quercetin supplementation reduced all pathologic changes in mice with rhinovirus-induced chronic obstructive pulmonary disease (COPD) and might prevent pulmonary disease progression in COPD [16]. Another experiment confirmed that quercetin could enhance ligand-induced senescent idiopathic pulmonary fibrosis fibroblast apoptosis and reduce lung fibrosis in vivo [17]. Luteolin has been shown to improve experimental pulmonary fibrosis in vivo and in vitro [18] and attenuate acute lung injury in experimental mouse models [19,20]. Isorhamnetin protects mice from acute lung injury by suppressing inflammation [21] and prevents bleomycin-induced pulmonary fibrosis by inhibiting endoplasmic reticulum stress and epithelial-mesenchymal transformation [22].

To further check the anti−COVID-19 mechanism of three active phytocompounds, the network pharmacology tool was used to analyze the targets of the phytocompounds, the cell signal transduction pathways that might be involved in the regulation, and the potential pharmacologic mechanism. SARS and viral pneumonia were used as reference diseases. Almost half of the targets of each phytocompound intersected with the targets of SARS and viral pneumonia. The results of network pharmacologic analysis indicated that the targets of the three plant compounds might provide resistance against COVID-19 through 12 overlapping pathways, 3 of which (the small-cell lung cancer pathway, non-small-cell lung cancer, and tuberculosis) acted directly on the lungs. Because of the mechanisms of immune resistance and tissue resilience, the innate immune system pathway plays an important role in worsened pneumonia in a subset of patients [23]. An earlier study confirmed that flavonoids inhibited cell proliferation and induced apoptosis and autophagy through downregulation of PI3Kγ-mediated PI3K-Akt signaling pathway [24]. It has been found in many studies that drugs can suppress the inflammatory response through the PI3K-Akt signaling pathway [25–28] Tumor necrosis factor drives its own release as well as that of other proinflammatory cytokines (e.g., interleukin [IL]-1β and IL-6) [29,30] and participates in the systemic inflammatory response. It is one of the cytokines that contribute to the acute phase response. A study revealed that spatial heterogeneity of the T–cell receptor repertoire reflected the mutational landscape in lung cancer [31], and T–cell-targeted immunotherapy has been increasingly applied to

the treatment of non-small-cell lung cancer [32]. The enrichment of these pathways from network pharmacology confirmed the hypothesis that quercetin, luteolin, and isorhamnetin were natural and effective anti−COVID-19 supplements.

Given the predictability of the virtual screening results, further in vitro and in vivo experiments are needed to verify the results of this study if possible, so as to provide the experimental basis for the development of natural anti−COVID-19 supplements. Unfortunately, many experiments could not be carried out due to the current epidemic, so this research was largely based on previous studies and theoretical analysis. Additionally, due to the human metabolism, it is difficult to achieve the concentration of active ingredients in food that is present in clinical drugs, and the preventive effect of food may be relatively weak.

Conclusion

Red wine, Chinese hawthorn, and blackberry are rich dietary sources of polyphenols with reported health benefits. They are recommended as preventive supplements because they contain three anti−COVID-19 phytocompounds (i.e., quercetin, luteolin, and isorhamnetin). The analysis of the anti−COVID-19 mechanism of these three compounds by internet pharmacology tools identifies several key pathways, which theoretically confirms the hypothesis that berries are a natural anti−COVID-19 supplement.

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