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Original Article

Exploration of Hanshi Zufei prescription for treatment of COVID-19 based on network pharmacology

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ABSTRACT

Objective: Network pharmacology combines drug and disease targets with biological information networks based on the integrity and systematicness of the interactions between drugs and disease targets. This study aims to explore the molecular basis of Hanshi Zufei formula for treatment of COVID-19 based on network pharmacology and molecular docking techniques.

Methods: Using TCMSP, the chemical constituents and molecular targets of *Atractylodis Rhizoma*, *Citri Reticulatae Pericarpium*, *Magnoliae Officinalis Cortex*, *Pogostemonis Herba*, *Tsaoko Fructus*, *Ephedrae Herba*, *Notopterygii Rhizoma* et *Radix*, *Zingiberis Rhizoma Recens*, and *Arecae Semen* were investigated. The predicted targets of novel coronavirus were screened using the NCBI and GeneCards databases. To further screen the drug-disease core targets network, the corresponding target proteins were queried using multiple databases (Biogrid, DIP, and HPRD), a protein interaction network graph was constructed, and the network topology was analyzed. The molecular docking studies were also performed between the network's top 15 compounds and the coronavirus (SARS-CoV-2) 3CL hydrolytic enzyme and angiotensin conversion enzyme II (ACE2).

Results: The herb-active ingredient-target network contained nine drugs, 86 compounds, and 49 drug-disease targets. Gene ontology (GO) enrichment analysis resulted in 1566 GO items ($P < 0.05$), among which 1438 were biological process items, 35 were cell composition items, and 93 were molecular function items. Fourteen signal pathways were obtained by enrichment screening of the KEGG pathway database ($P < 0.05$). The molecular docking results showed that the affinity of the core active compounds with the SARS-CoV-2 3CL hydrolase was better than for the other compounds.

Conclusion: Several core compounds can regulate multiple signaling pathways by binding with 3CL hydrolase and ACE2, which might contribute to the treatment of COVID-19.

1. Introduction

The 2019 coronavirus disease (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which can be transmitted by respiratory droplets, close contact, and aerosols, and is characterized by strong infectiousness, rapid transmission, and rapid disease progression (Chinese preventive medicine association COVID-19 prevention and control expert group, 2020; Qin et al., 2020). The National Health Commission lists SARS-CoV-2 as a class B infectious disease and adopts the prevention and control measures for class A infectious diseases (Qin et al., 2020). In late 2019, COVID-19 broke out around the world. By April 20, 2020, 2 318 457 cases had been confirmed abroad, and the number of confirmed cases increased. The National Health Committee has published seven editions of the COVID-19 medical scheme, including Western medicine treatments with experimental drugs, such as α-interferon, lopinavir, ritonavir, and a series of respiratory and circulatory support therapies. Because the curative effect of this approach is poor and is associated with side effects, the third edition scheme included traditional Chinese medicines

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(TCMs). Many TCM prescriptions have been applied in COVID-19 patients, and treatments integrating traditional Chinese and Western medicine have been remarkably successful (Dong, Wang, & Li, 2020).

According to the COVID-19 protocol (trial version 7) released by the National Health Commission, patients with different symptoms will be treated differently. The clinical manifestations were low fever, non-fluttery body heat or no heat, dry cough, less sputum, tiredness, fatigue, chest tightness, vomiting and loose stool, with a tongue coating of slightly light or slightly red, moss of thin white or white greasy, pulse of moisten moisten. The recommended prescription (hereinafter referred to as "Hanshi Zufei prescription") is: *Atractylodis Rhizoma* (15 g), *Citri Reticulatae Pericarpium* (10 g), *Magnoliae Officinalis Cortex* (10 g), *Pogostemonis Herba* (10 g), *Tsaoko Fructus* (6 g), *Ephedrae Herba* (6 g), *Notopterygii Rhizoma* et *Radix* (10 g), *Zingiberis Rhizoma Recens* (10 g), and *Arecae Semen* (10 g).

This prescription is a modification of the traditional Chinese medicine prescriptions Pingweisan and Dayuanyin, which have been used during disease outbreaks (Wen & Zhang, 2020). *Atractylodis Rhizoma*, which is spicy, bitter, and warm, eliminates dampness to strengthen the spleen; *Magnoliae Officinalis Cortex* can aromatize turbidness, dehumidify and regulate *qi*; *Citri Reticulatae Pericarpium* can regulate *qi* and stomach dryness and dampening up the spleen; *Pogostemonis Herba* can prevent vomiting; *Tsaoko Fructus* have anti-retching and anti-evil effects; *Ephedrae Herba* can alleviate sweat and cold, relieve lung asthma, and subside swelling; *Notopterygii Rhizoma* et *Radix* has the effect of dispelling cold, dehumidification, and analgesia; *Zingiberis Rhizoma Recens* have the effects of dispelling cold and stopping vomiting, phlegm, and cough; *Arecae Semen* scatters damp evil, dissolves phlegm, and loosens knots. This prescription had been remarkably effective in treating COVID-19 (Peng, He, Sun, Wang, & Huang, 2019; Shi et al., 2019; Zhao, Li, & Xie, 2020).

Network pharmacology is a systematic study to explore the interactions between active ingredients, targets, and diseases (Li & Zhang, 2013; Xu et al., 2020; Zhang, Hong, Chen, Zhou, & Li, 2019; Zhang, Liang, Wang, & Yang, 2019). Here we used network pharmacology and molecular docking techniques to explore the molecular basis for the Hanshi Zufei formula to treat COVID-19.

2. Materials and methods

2.1. Targets of active ingredients and novel coronavirus database building

Using *Atractylodis Rhizoma*, *Citri Reticulatae Pericarpium*, *Magnoliae Officinalis Cortex*, *Pogostemonis Herba*, *Tsaoko Fructus*, *Ephedrae Herba*, *Notopterygii Rhizoma* et *Radix*, *Zingiberis Rhizoma Recens*, and *Arecae Semen* as the nine TCM keywords, using TCMSP (https://lsp.nwu.edu.cn/TCMSP. PHP) retrieval, the active ingredients and corresponding targets were screened to identify those with oral bioavailability (OB) >30% and drug-likeness (DL) >0.18. Based on the NCBI (https://www.ncbi.nlm.nih.gov/) and GeneCards (https://www.genecards.org/) databases, using "novel coronavirus" as keywords, genes associated with COVID-19 were searched (Ru et al., 2014; Zeng, Li, Siu, Jin, & Wu, 2019; Zhou, Wang, Xiang, Tong, & Chen, 2019).

2.2. Construction of a herb-active ingredient-target regulation network

The corresponding targets of the selected active ingredients were annotated to obtain the gene symbols. The genes associated with COVID-19 in the GeneCards database were modified. The drug target genes were mapped to the disease genes, and common targets were obtained and visualized using a Venn diagram (Bu, Su, Zou, Meng, & Wang, 2019; Shawky, 2019; Wei, Zhou, Niu, Zhang, & Zhao, 2019). The shared drug and disease targets were used to construct the herb-active ingredient-target regulatory network using Cytoscape software (version 3.7.1).

2.3. Construction of a target protein interaction network (PPI)

Using the BisoGenet plug-in of Cytoscape (3.7.1), the protein type "Homo sapiens" was selected to construct a protein interaction network. Then, we used the CytoNCA plug-in for network topology analysis. Generally speaking, Degree centrality (DC) and Betweenness centrality (BC) are of great significance to the whole network when they are greater than the median value. To generate a more intuitive drug-disease core targets network and mine the information of biological value, this study selected DC > 61 and BC > 600 as screening criteria (Szklarczyk et al., 2015; Szklarczyk et al., 2017).

2.4. Enrichment analysis of GO and KEGG pathways

The Bioconductor software package was used to perform enrichment analyses of gene ontology (GO), molecular function (MF), biological process (BP), and cell composition (CC). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were used to identify common targets. Hits with *P*-values $\leq$0.05 were retained and used to construct the GO histogram and KEGG signal pathway bubble map. Finally, Cytoscape software was used to build the common target-signal pathway network diagram (Li, Guo, & Sun, 2019; Ning et al., 2017; Singh, Vennila, Snijesh, George, & Sunny, 2016).

2.5. Molecular docking

We used ZINC (https://http://zinc.docking.org/) to confirm the database name of the compound and its molecular weight while also retrieving the compound's three-dimensional structural formula (mol2 format), then used AutoDock to convert to *PDBQT format. The 3D structure files of SARS-CoV-2 3CL hydrolase and Angiodensin Converting Enzyme II (ACE2) were downloaded from the RSCB PDB database (https://www.rcsb.org/), and then AutoDock 4.2.6 (http://autodock. Scripps. edu/news/autodock42.6) was used to remove water, and to perform the hydrotreating and other operations. The result was saved as a *PDBQT format file for later use. Ultimately, for the protein analysis, we used the AutoDock docking operation, then used the Pymol compounds dock (Wang et al., 2020; Zheng, Mo, Wu, Guo, & Bao, 2019; Zong, Yao, Shan, & Wang, 2020).

3. Results

3.1. Active ingredients and targets of nine herbs in prescription

The TCMSP database was used to retrieve the active ingredients contained in nine traditional Chinese medicines, and the parameters of OB > 30% and DL > 0.18 were used as the screening conditions. We found that 86 active ingredients were obtained: *Arecae Semen* (n = 8), *Atractylodis Rhizoma* (n = 9), *Tsaoko Fructus* (n = 8), *Citri Reticulatae Pericarpium* (n = 5), *Pogostemonis Herba* (n = 11), *Magnoliae Officinalis Cortex* (n = 2), *Ephedrae Herba* (n = 23), *Notopterygii Rhizoma* et *Radix* (n = 15), and *Zingiberis Rhizoma Recens* (n = 5) (Table 1). A further 228 related targets were obtained by predicting "related targets" and removing duplications.

Table 1
Basic information on active ingredients.

Herbs	MOL ID	Compounds	OB (%)	DL
Arecae Semen	MOL000004	Procyanidin B1	67.87	0.66
	MOL000073	ent-Epicatechin	48.96	0.24
	MOL001749	ZINC03860434	43.59	0.35
	MOL002032	DNOP	40.59	0.40
	MOL002372	(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	33.55	0.42
	MOL010482	WLN: 6OVR BVO6	43.74	0.24
	MOL010485	EPA	45.66	0.21
	MOL010489	Resivit	30.84	0.27
Atractylodis Rhizoma	MOL000179	2-Hydroxyisoxypropyl-3-hydroxy-7-isopentene-2,3-dihydrobenzofuran-5-carboxylic	45.20	0.20
	MOL000186	Stigmasterol 3-*O*-*β*-*D*-glucopyranoside	43.83	0.76
	MOL000188	3*β*-acetoxyatractylone	40.57	0.22
	MOL000184	NSC63551	39.25	0.76
	MOL000085	*β*-Daucosterol	36.91	0.75
	MOL000088	*β*-Sitosterol 3-*O*-glucoside	36.91	0.75
	MOL000092	Daucosterin	36.91	0.76
	MOL000094	Daucosterol	36.91	0.76
	MOL000173	Wogonin	30.68	0.23
Tsaoko Fructus	MOL000074	(4E,6E)-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one	67.92	0.24
	MOL000096	(−)-Catechin	49.68	0.24
	MOL000073	ent-Epicatechin	48.96	0.24
	MOL000098	Quercetin	46.43	0.28
	MOL000085	*β*-Daucosterol	36.91	0.75
	MOL000088	*β*-Sitosterol 3-*O*-glucoside	36.91	0.75
	MOL000092	Daucosterin	36.91	0.76
	MOL000094	Daucosterol	36.91	0.76
Citri Reticulatae Pericarpium	MOL005815	Citromitin	86.90	0.51
	MOL005828	Nobiletin	61.67	0.52
	MOL004328	Naringenin	59.29	0.21
	MOL005100	5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one	47.74	0.27
	MOL000359	Sitosterol	36.91	0.75
Pogostemonis Herba	MOL005890	Pachypodol	75.06	0.40
	MOL005911	5-Hydroxy-7,4′-dimethoxyflavanon	51.54	0.27
	MOL005921	Quercetin 7-*O*-*β*-*D*-glucoside	49.57	0.27
	MOL000098	Quercetin	46.43	0.28
	MOL002879	Diop	43.59	0.39
	MOL005922	Acanthoside B	43.35	0.77
	MOL005918	Phenanthrone	38.70	0.33
	MOL005884	Patchoulan 1,12-diol	38.17	0.25
	MOL005916	Irisolidone	37.78	0.30
	MOL005573	Genkwanin	37.13	0.24
	MOL005923	3,23-Dihydroxy-12-oleanen-28-oic acid	30.86	0.86
Magnoliae Officinalis Cortex	MOL005970	Eucalyptol	60.62	0.32
	MOL005980	Neohesperidin	57.44	0.27
Ephedrae Herba	MOL005190	Eriodictyol	71.79	0.24
	MOL004328	Naringenin	59.29	0.21
	MOL010788	Leucopelargonidin	57.97	0.24
	MOL004576	Taxifolin	57.84	0.27
	MOL000492	(+)-Catechin	54.83	0.24
	MOL000098	Quercetin	46.43	0.28
	MOL000449	Stigmasterol	43.83	0.76
	MOL011319	Truflex OBP	43.74	0.24
	MOL001494	Mandenol	42.00	0.19
	MOL000422	Kaempferol	41.88	0.24
	MOL005842	Pectolinarigenin	41.17	0.30
	MOL004798	Delphinidin	40.63	0.28
	MOL007214	(+)-Leucocyanidin	37.61	0.27
	MOL005043	Campest-5-en-3*β*-ol	37.58	0.71
	MOL005573	Genkwanin	37.13	0.24
	MOL000358	*β*-Sitosterol	36.91	0.75
	MOL001771	Poriferast-5-en-3*β*-ol	36.91	0.75
	MOL000006	Luteolin	36.16	0.25
	MOL001755	24-Ethylcholest-4-en-3-one	36.08	0.76
	MOL002823	Herbacetin	36.07	0.27
	MOL001506	Supraene	33.55	0.42
	MOL002881	Diosmetin	31.14	0.27
	MOL010489	Resivit	30.84	0.27
Notopterygii Rhizoma et *Radix*	MOL011971	Diversoside	67.57	0.31
	MOL011975	Notoptol	62.97	0.48
	MOL004792	Nodakenin	57.12	0.69
	MOL001942	Isoimperatorin	45.46	0.23
	MOL001951	Bergaptin	41.73	0.42
	MOL011969	Demethylfuropinnarin	41.31	0.21
	MOL011963	8-Geranoxy-5-methoxypsoralen	40.97	0.50
	MOL002644	Phellopterin	40.19	0.28

Table 1 (*continued*)

Herbs	MOL ID	Compounds	OB (%)	DL
	MOL000359	Sitosterol	36.91	0.75
	MOL000358	β-Sitosterol	36.91	0.75
	MOL001941	Ammidin	34.55	0.22
	MOL011968	Coumarin, glycoside	33.07	0.78
	MOL001956	Cnidilin	32.69	0.28
	MOL011962	6′-Feruloylnodakenin	32.02	0.67
	MOL002881	Diosmetin	31.14	0.27
Zingiberis Rhizoma Recens	MOL006129	6-Methylgingediacetate2	48.73	0.32
	MOL008698	Dihydrocapsaicin	47.07	0.19
	MOL000449	Stigmasterol	43.83	0.76
	MOL000358	β-Sitosterol	36.91	0.75
	MOL001771	Poriferast-5-en-3β-ol	36.91	0.75

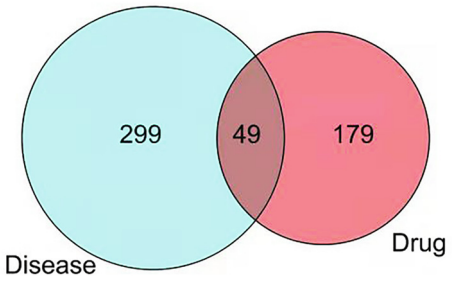

Fig. 1. Venn figure of candidate targets of active ingredients and novel coronavirus.

3.2. Prediction of potential targets of novel coronavirus

A total of 394 potential targets of novel coronavirus were collected through NCBI and GeneCards databases, and 46 duplicates were eliminated. A total of 348 novel coronavirus-related targets were obtained.

3.3. Analysis of herb-active ingredient-target regulation network

The 228 active ingredient targets predicted from the TCMSP database and 348 novel coronavirus disease targets were plotted as a Venn diagram, and 49 overlapping targets were obtained (Fig. 1), which are the targets related to the active ingredients of traditional Chinese medicine acting on diseases.

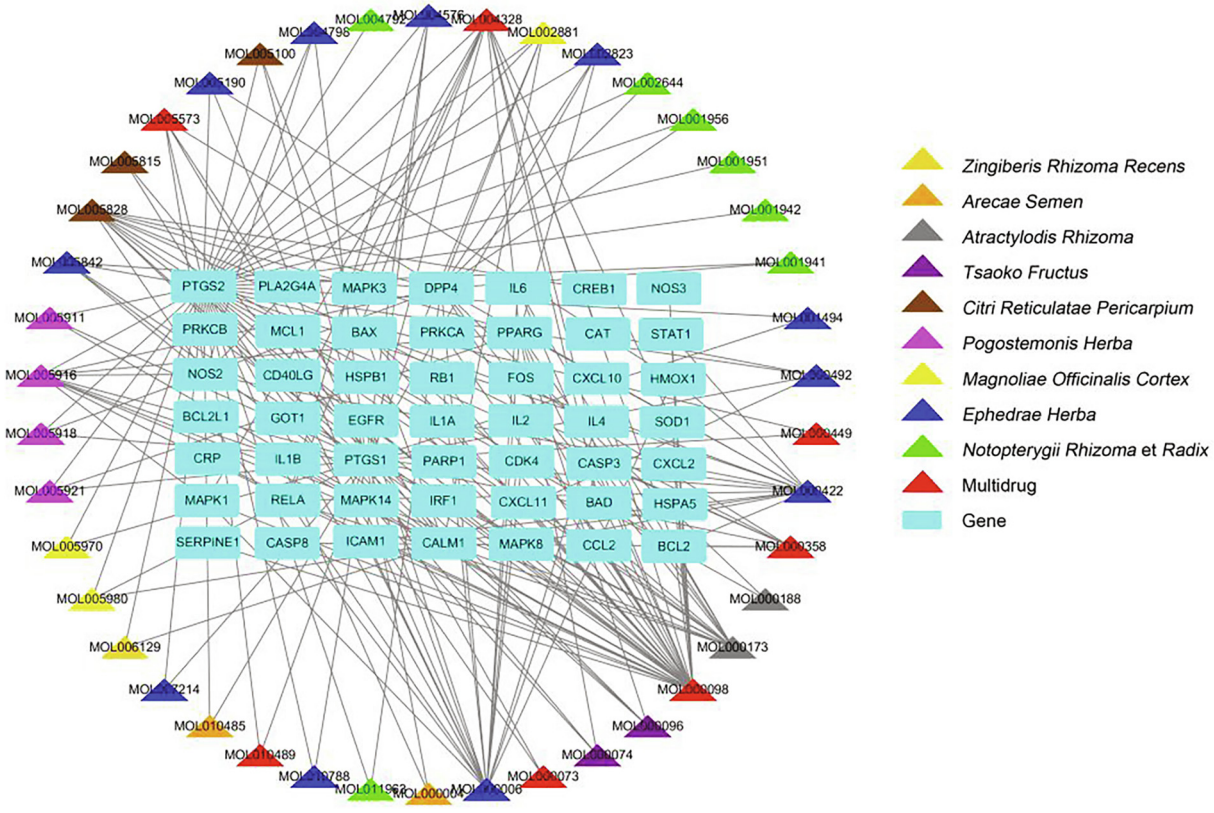

Fig. 2. Herb-active ingredient-target regulation network.

Cytoscape software was used to construct a herbs-active ingredients-targets network. The network has 91 nodes and 212 edges, among which the light blue rectangular nodes represent overlapping targets (Gene), the triangles represent compounds, and different colours represent different TCMs. Each edge represents the interaction between compounds and targets (Fig. 2).

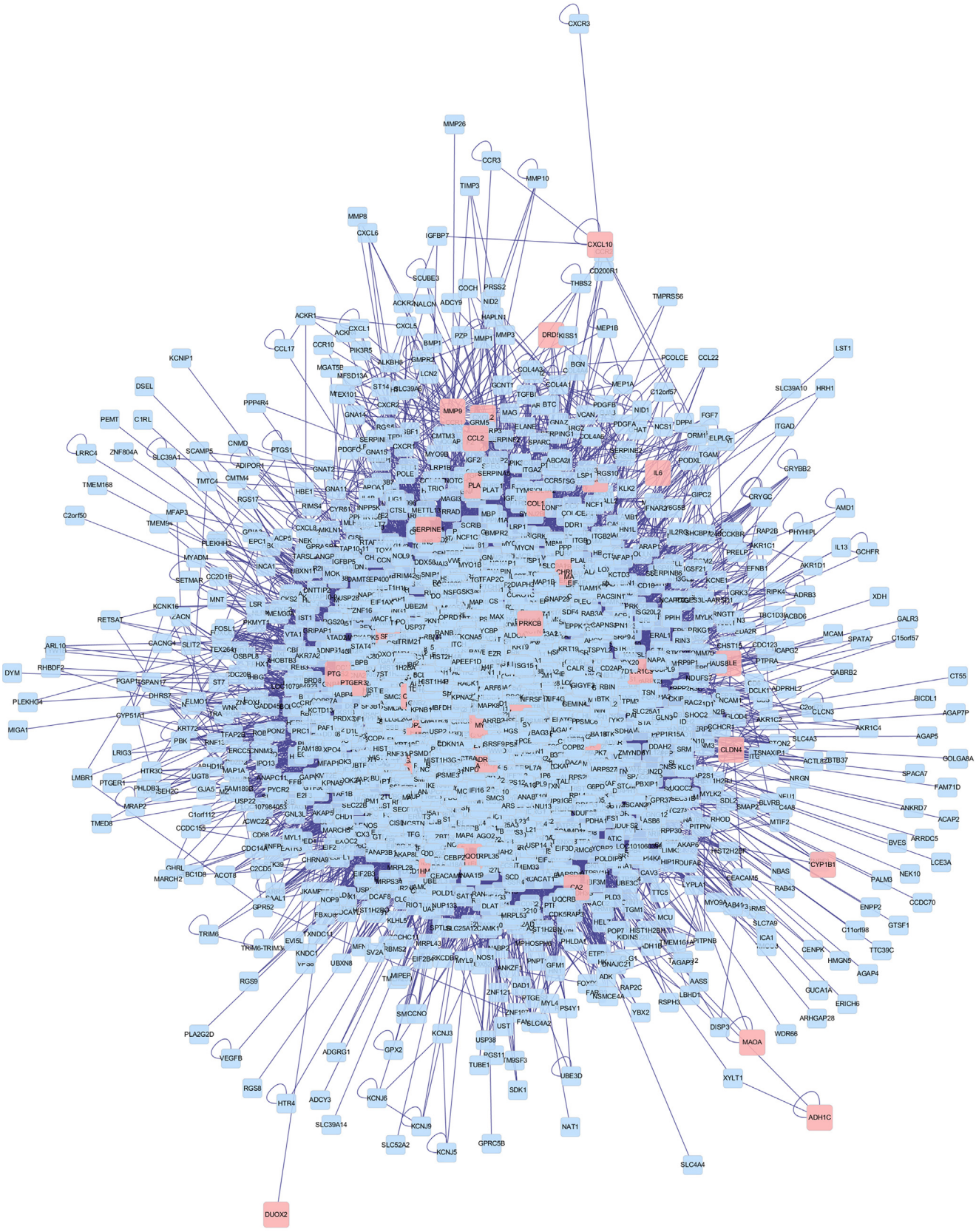

Fig. 3. PPI network diagram.

According to degree ranking, the core active ingredients were quercetin (MOL000098, value = 36), luteolin (MOL000006, value = 18), kaempferol (MOL000422, value = 14), wogonin (MOL000173, value = 14), naringenin (MOL004328, value = 12), nobiletin (MOL005828, value = 11), and irisolidone (MOL005916, value = 10).

3.4. Target protein interaction network (PPI) construction

The common target information was imported into Cytoscape (3.7.1) software. The network relationship between target proteins was constructed using BisoGenet to obtain a PPI network (Fig. 3), including 2 148 proteins and 47 330 interrelation channels. The network topology analysis was performed on 2148 proteins,

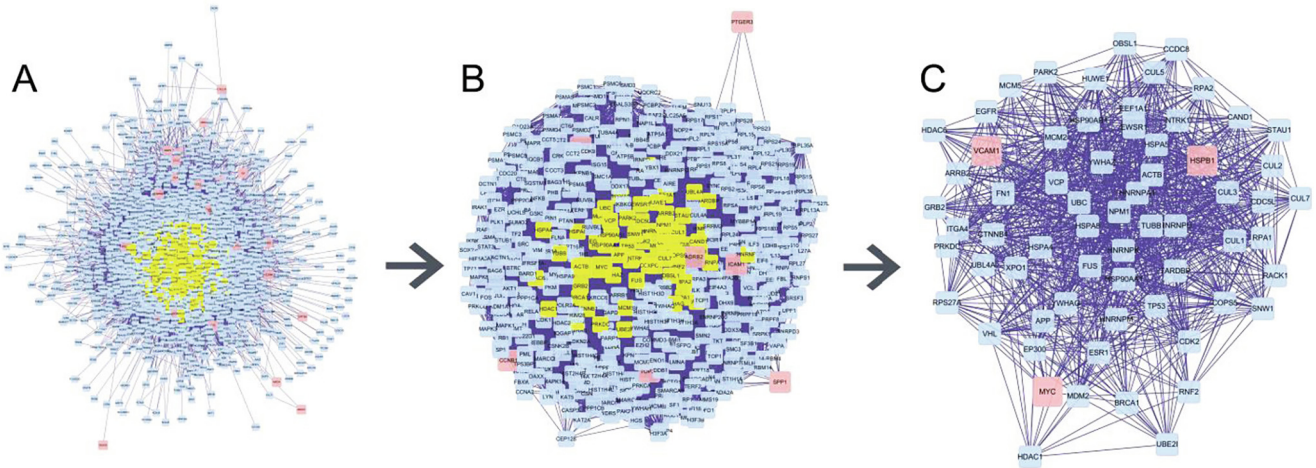

Fig. 4. PPI topology analysis. A: PPI network of 2148 targets; B: PPI network screened by DC; C: PPI network of 65 targets screened by BC.

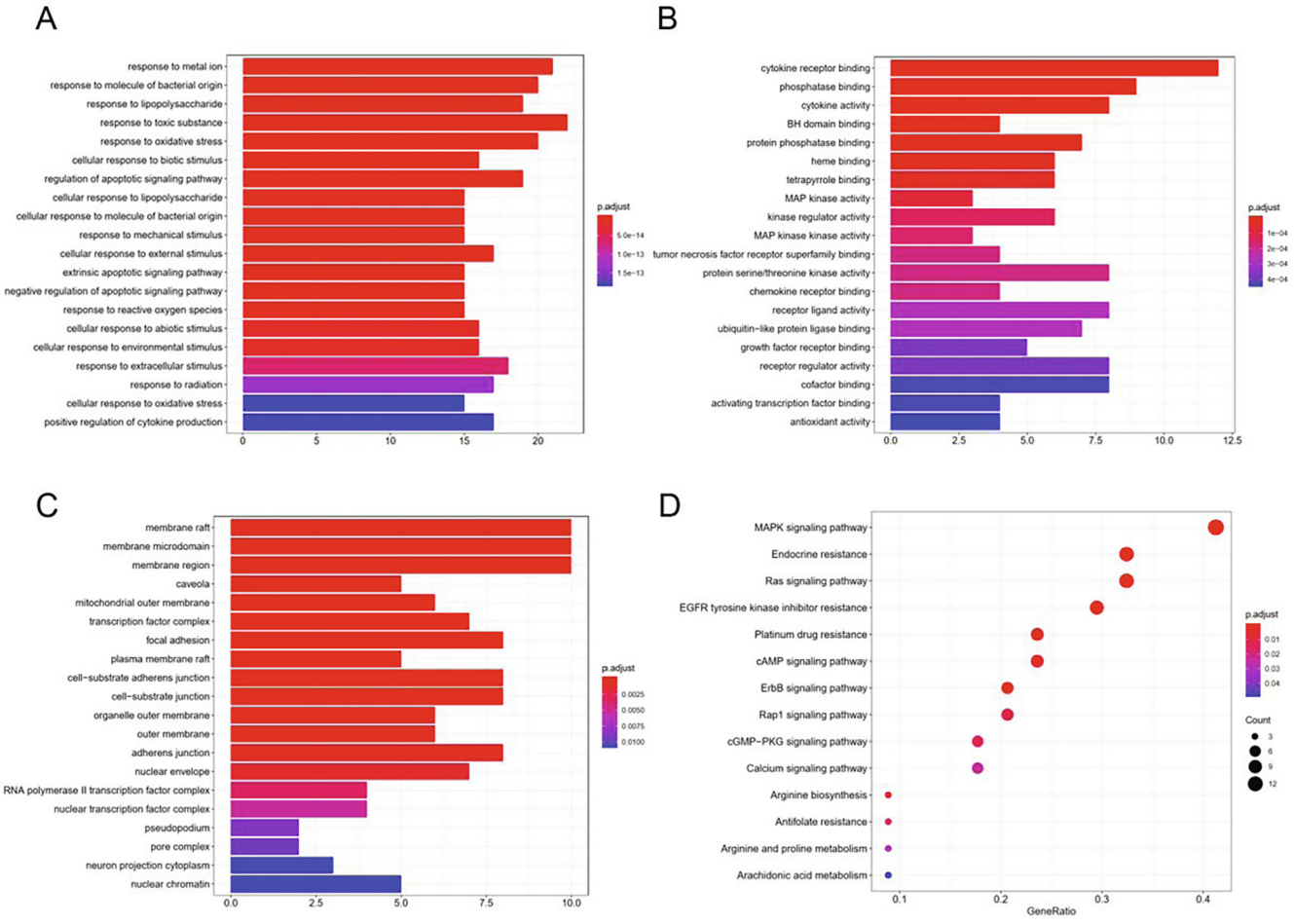

Fig. 5. Results of enrichment analyses. A: GO biological process; B: GO molecular function; C: GO cell composition, D: KEGG enrichment result.

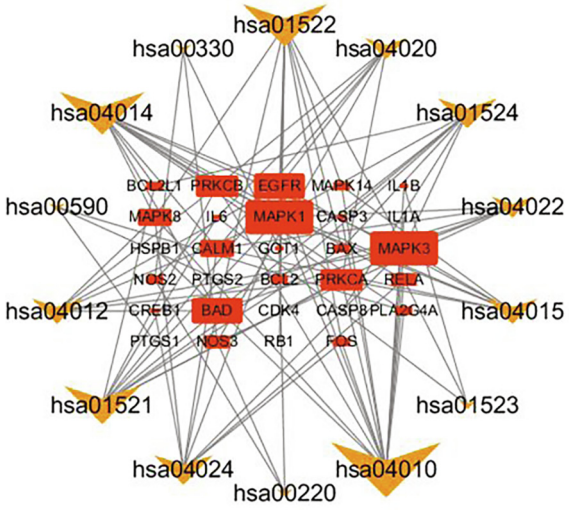

Fig. 6. Target-pathway network.

including DC and BC (Fig. 4A). When the DC value was >61 (Fig. 4B) and BC value was >600, 65 target proteins and 1006 interaction relationships were obtained (Fig. 4C). The top six target proteins were NTRK1, CUL3, TP53, FN1, MCM2, and CDK2.

3.5. Enrichment analysis of target GO and KEGG pathways

GO and KEGG enrichment analyses were carried out on 49 common targets. According to the retaining results ($P < 0.05$), the first 20 items were visualized and analyzed to generate a histogram and bubble graph (Ma et al., 2020). The vertical coordinates of the bubble diagram indicate the KEGG name, and the horizontal coordinates indicate the gene proportion. The co-acting targets in the biological process (BP) are mainly response to toxic substances, response to the metal ion, and response to oxidative stress (Fig. 5A). The common targets in the molecular function (MF) are mainly cytokine receptor binding, phosphatase binding, cytokine activity, and other processes (Fig. 5B). The common targets in cell composition (CC) mainly involve membrane raft, membrane microdomain, and membrane region (Fig. 5C). Through KEGG pathway enrichment analysis ($P < 0.05$), the common targets were mainly concentrated in the MAPK signalling pathway, endocrine resistance, Ras signalling pathway, and other relevant signalling pathways (Fig. 5D).

The screened targets and pathways were imported into Cytoscape software to construct the targets-KEGG network diagram (Fig. 6). Red represents the targets, and yellow represents the KEGG pathway. The results showed that the pathways with many adjacent nodes included the MAPK signalling pathway, endocrine resistance, and Ras signalling pathway, and the targets include MAPK1, MAPK3, BAD, and EGFR.

3.6. Molecular docking

The molecular docking results showed that the minimum binding energy between the core active ingredient (herbacetin, genkwanin and wogonin) and SARS-CoV-2 3CL hydrolase were below −5 kcal/mol, indicating that they had good binding activity and can form stable binding conformation. Molecular docking of the core compound with ACE2 (PDB ID: 1R42) was performed, and the results were shown in Fig. 7 and Table 2.

4. Discussion

Since ancient times, TCM has played an important role in the prevention and treatment of epidemic infectious diseases, such as smallpox, plague, meningitis, haemorrhagic fever, and swine flu, and many effective methods have been widely used in clinical practice (Huang, Wang, Xu, & Liu, 2020). In Dayuanyin, *Arecae Semen*, *Tsaoko Fructus*, and *Magnoliae Officinalis Cortex* play a key role in treating plague. Pingwei powder is widely used to dry

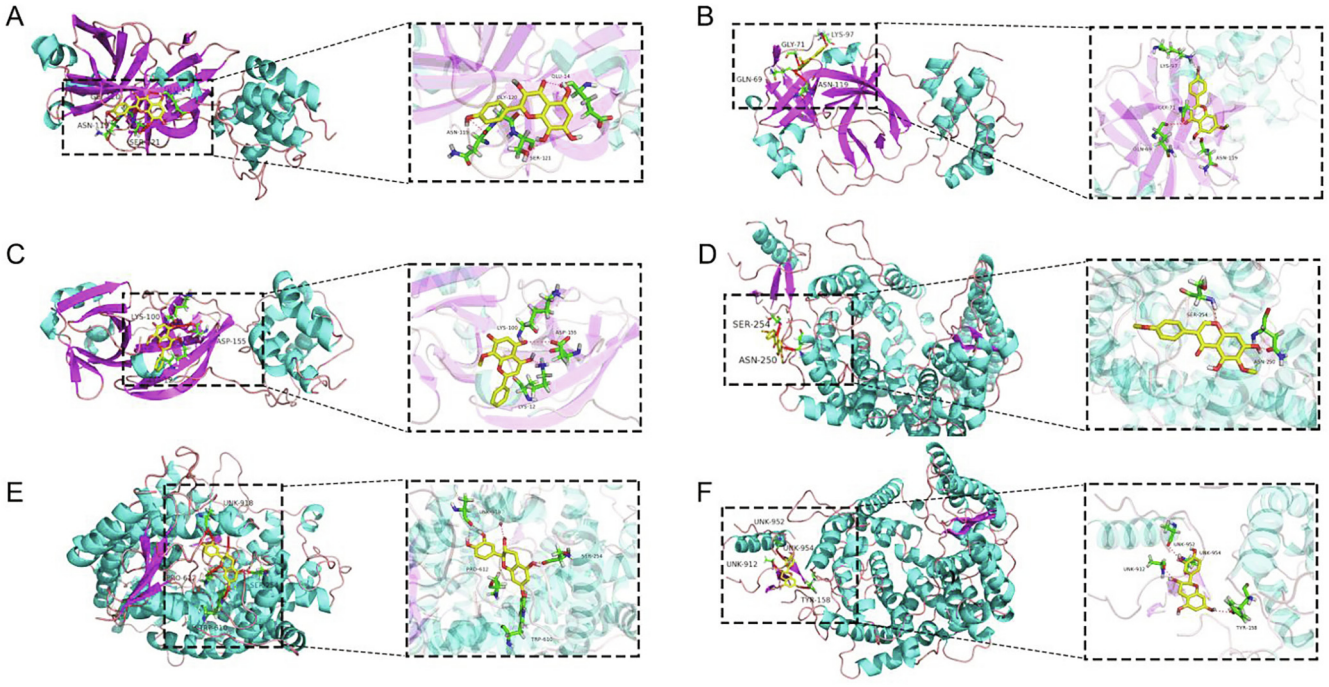

Fig. 7. Molecular docking pattern of SARS-CoV-2 3CL hydrolase, ACE2 and core compounds. A: Herbacetin-3CL hydrolase; B: Genkwanin-3CL hydrolase; C: Wogonin-3CL hydrolase; D: irisolidone-ACE2; E: (−)-catechin-ACE2; F: (+)-catechin-ACE2.

Table 2
Binding affinity of core compounds with SARS-CoV-2 3CL hydrolase and ACE2.

Compounds	Chemical formula	Relative molecular mass	Binding energy with SARS-CoV −2 3CL hydrolase (kJ/mol)	Binding energy with ACE2 (kJ/mol)
Quercetin	$C_{15}H_{10}O_7$	302.24	−3.66	−3.51
Luteolin	$C_{15}H_{10}O_6$	286.23	−3.87	−1.96
Kaempferol	$C_{15}H_{10}O_6$	286.23	−3.62	−3.06
Wogonin	$C_{16}H_{12}O_5$	284.27	−4.28	−3.72
Naringenin	$C_{15}H_{12}O_5$	272.25	−4.05	−2.69
Nobiletin	$C_{21}H_{22}O_8$	402.39	−2.93	−1.32
Irisolidone	$C_{17}H_{14}O_6$	314.00	−3.18	−4.16
β-Sitosterol	$C_{29}H_{50}O$	414.71	−4.15	−3.41
Pectolinarigenin	$C_{17}H_{14}O_6$	314.00	−3.72	−3.13
Diosmetin	$C_{16}H_{12}O_6$	300.26	−3.59	−2.74
Genkwanin	$C_{16}H_{12}O_5$	284.27	−4.45	−3.75
Taxifolin	$C_{15}H_{12}O_7$	304.25	−3.34	−3.42
(+)-Catechin	$C_{15}H_{14}O_6$	290.27	−3.05	−4.02
Herbacetin	$C_{15}H_{10}O_7$	302.24	−4.73	−3.20
(−)-Catechin	$C_{15}H_{14}O_6$	290.27	−3.12	−4.11

dampness, promote *qi*, and regulate the stomach. In the Hanshi Zufei formula, Dayuanyin and Pingwei powder have been combined to dialectically add and reduce the drug flavour, and the drug treatment has a good effect from the comprehensive and holistic concept of traditional Chinese medicine.

4.1. Potential targets

This study screened 228 potential drug targets, 49 overlapping targets and 15 core compounds (quercetin, luteolin, kaempferol, wogonin, naringenin, nobiletin, irisolidone, β-sitosterol, pectolinarigenin, diosmetin, genkwanin, taxifolin, (+)-catechin, herbacetin, and (−)-catechin). Twelve of these are flavonoids, (+)-catechin and (−)-catechin are phenols, and β-sitosterol is a phytosterol (Wang et al., 2020). Flavonoids have anti-inflammatory, antibacterial, antiviral, and immunological effects, and these activities might be related to their anti-free radical or anti-oxidation activities (Ma & Wu, 2013). According to PPI network topological analysis, the drug-disease core targets network was screened, such as NTRK1, CUL3, TP53, and FN1. Among them, NTRK1 is the medium nerve growth factor for promoting survival signal transduction, and it is also a known oncogene that has been generally changed in human cancer, which can be used as a drug target (Kim et al., 2015). CUL3 is a ubiquitinated ligase E3 protein, which belongs to the Cullin protein family. Deletion or mutation in animal models has a lethal embryonic phenotype. The TP53 gene encodes a tumour suppressor protein containing transcriptional activation, DNA binding, and oligonucleotide domains, which regulates the expression of target genes in response to various cellular stress responses, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, and metabolic changes. The FN1 gene encodes fibronectin, which is involved in cell adhesion and migration, including embryogenesis, wound healing, coagulation, host defence, and metastasis.

4.2. Pathway analysis

According to the GO enrichment analysis results, the main enrichment items in biological processes are a response to toxic substances, response to metal ions, and response to oxidative stress. According to our KEGG enrichment analysis results, the main pathways involved in the treatment process include MAPK signalling, endocrine resistance, and Ras signalling pathways. The targets were MAPK1, MAPK3, BAD, and EGFR. Among them, the *BAD* gene is an important regulatory molecule of cell apoptosis, and the *EGFR* gene is a receptor for epithelial growth factor cell proliferation and signal transduction. Mutations or overexpression of these genes will generally cause tumours (He & Xue, 2007; Wang, 2019). The MAPK signalling pathway regulates a variety of important cellular physiological/pathological processes, including cell growth, differentiation, stress adaptation to the environment, and inflammatory response, and plays a key role in gene expression regulation and cytoplasmic functional activities (Chen, Wang, Wang, & Cao, 2011). The above targets and pathways play a key role in treating common COVID-19, but further validation studies are needed.

4.3. Molecular docking analysis

The results of this study were verified by network pharmacology and the guidance of TCM. Our network pharmacology analysis indicates that the 15 prescription compounds with the highest score are all from herbs with warm nature and spicy flavours, such as *Pogostemonis Herba*, *Tsaoko Fructus*, and *Ephedrae Herba*. These core compounds were used for molecular docking with SARS-CoV-2 3CL hydrolase. All of them could bind to SARS-CoV-2 3CL hydrolase, and the binding energies were lower for herbacetin, genkwanin, and wogonin. It has been proposed that both SARS-CoV-2 and SARS-CoV infect humans by binding S-Protein to ACE2 (Tang, Li, Xu, & Shen, 2020). Therefore, molecular docking of the core compound with ACE2 was conducted in this study, and the binding energies of irisolidone, (−)-catechin, and (+)-catechin were lower than other core compounds. These results suggest that the core compounds [herbacetin, genkwanin, wogonin, irisolidone, (−)-catechin, and (+)-catechin] are potentially useful in the treatment of COVID-19 patients (Yao et al., 2020).

5. Conclusion

Our network pharmacology and molecular docking approaches show that several core compounds (herbacetin, genkwanin, and wogonin) can regulate the MAPK signalling pathway, endocrine resistance, and the Ras signalling pathway by binding with the SARS-CoV-2 3CL hydrolase and ACE2 on multiple targets (MAPK1, MAPK3, BAD, and EGFR), which might play a therapeutic role in COVID-19 patients. In summary, using a theory and network prediction approach, this study describes the roles and mechanisms of the Hanshi Zufei formula on COVID-19 in multiple components, multiple targets, and multiple pathways to provide guidance and ideas for further research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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