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Identifying potential treatments of COVID-19 from Traditional Chinese Medicine (TCM) by using a data-driven approach

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ABSTRACT

Ethnopharmacological relevance: Traditional Chinese Medicine (TCM) has been widely used as an approach worldwide. Chinese Medicines (CMs) had been used to treat and prevent viral infection pneumonia diseases for thousands of years and had accumulated a large number of clinical experiences and effective prescriptions.

Aim of the study: This research aimed to systematically excavate the classical prescriptions of Chinese Medicine (CM), which have been used to prevent and treat Pestilence (Wenbing, Wenyi, Shiyi or Yibing) for long history in China, to obtain the potential prescriptions and ingredients to alternatively treat COVID-19.

Materials and methods: We developed the screening system based on data mining, molecular docking and network pharmacology. Data mining and association network were used to mine the high-frequency herbs and formulas from ancient prescriptions. Virtual screening for the effective components of high frequency CMs and compatibility Chinese Medicine was explored by a molecular docking approach. Furthermore, network pharmacology method was used to preliminarily uncover the molecule mechanism.

Results: 574 prescriptions were obtained from 96,606 classical prescriptions with the key words to treat "Warm diseases (Wenbing)", "Pestilence (Wenyi or Yibing)" or "Epidemic diseases (Shiyi)". Meanwhile, 40 kinds of CMs, 36 CMs-pairs, 6 triple-CMs-groups existed with high frequency among the 574 prescriptions. Additionally, the key targets of SARS-COV-2, namely 3CL hydrolase (Mpro) and angiotensin-converting enzyme 2(ACE2), were used to dock the main ingredients from the 40 kinds by the LigandFitDock method. A total of 66 compounds components with higher frequency were docked with the COVID-19 targets, which were distributed in 26 kinds of CMs, among which *Gancao* (Glycyrrhizae Radix Et Rhizoma), *HuangQin* (Scutellariae Radix), *Dahuang* (Rhei Radix Et Rhizome) and *Chaihu* (Bupleuri Radix) contain more potential compounds. Network pharmacology results showed that *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) CMs-pairs could also interact with the targets involving in immune and inflammation diseases.

Conclusions: These results we obtained probably provided potential candidate CMs formulas or active ingredients to overcome COVID-19. Prospectively, animal experiment and rigorous clinic studies are needed to confirm the potential preventive and treat effect of these CMs and compounds.

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1. Introduction

Corona Virus Disease 2019 (COVID-19), which is caused by a newly identified coronavirus SARS-COV-2, has been spread to more than 200 countries and regions around the world and posing significant threats to public health (Chen et al., 2020). Unfortunately, it is still raging with no effective drugs clinically approved. Given the severity of SARS-COV-2, it is critical to discovery and clinical application of specific drugs against SARS-COV-2 to alleviate the current epidemic situation. It is particularly important to screen possible blockers for the potential target proteins of virus by computational chemical biology techniques such as molecular docking ("dry method"research) in special cases such as the outbreak of SARS-COV-2 (Li et al., 2020a). This approach conducive to large-scale screening in a short period of time. It has been recommended two main proteins, 3C-like protease (3CLpro) and angiotensin-converting enzyme 2 (ACE2), could be used as available targets for screening drugs that inhibiting the replication and proliferation of SARS-COV-2, benefit from rapid sequencing of SARS-COV-2 coupled with molecular modelling based on the genomes of related viral proteins (Chen et al., 2020; Chai et al., 2020).

Chinese Medicines (CMs), a long history system of medicine with distinct features of theories and practices, has been used for thousands of years (Qiu, 2015). CMs prescriptions embody the principles of system theory, and act on multiple cellular targets in multiple pathways to exert therapeutic effects (Hou et al., 2016; Liao et al., 2018). COVID-19 belongs to the category of pestilence or epidemic in CM (Li et al., 2020). CMs had been used to treat and prevent viral infection pneumonia diseases for thousands of years and had accumulated a large number of clinical experience and effective prescription (Luo et al., 2019). In the "Diagnosis and Treatment Program for Corona Virus Disease 2019 (COVID-19)" issued by the National Health Commission of China, it is recommended to treat with CMs and had achieved good clinical effects. Thus, it is very significant to explore and mine experiences of CMs in treating of pestilence or epidemic diseases based on the abundant historical classics of CMs combined with modern medical research method. In the present study, data mining and association network were used to mine the high-frequency CMs and formulas from ancient prescriptions. Furthermore, molecular docking approach were used to explore binding rates between the main ingredients in high frequency CMs and the key targets of SARS-COV-2. Then, we preliminarily uncover molecular mechanism by a network pharmacology process (Fig. 1). These results are expected to provide referenced candidate CMs formulas or active compounds to overcome COVID-19.

2. Material and method

2.1. Data mining

2.1.1. Data sources

In our study, the *Dictionary of Traditional Chinese Medicine Prescriptions* (Peng, 1996) and *Pharmacopoeia of the People's Republic of China* (Pharmacopoeia Commission China, 2015) were used to screened the prescriptions containing "Warm diseases (Wenbing)", "Pestilence (Wenyi or Yibing)" or "Epidemic diseases (Shiyi)".

The data processing process included the following three steps: Firstly, the relevant prescriptions retrieved were inputted into the word document to obtain the original literature file; Secondly, key words of prescription information contained number, name, source, formula, efficacy, therapy, dosage form and number of natural medicines and ancient literature classified information were inputted into the Excel file; Finally, prescription data were standardized on the basis of *Pharmacopoeia of the People's Republic of China* (Peng, 1996) and the *Chinese Materia Medica* (Zhu, 1998). Then standardized data was imported to database for following data mining and association network analysis.

2.1.2. Data analyze and association network analysis

In our present study, frequency analysis method, association rule mining method and association knowledge network construction method were used to analyze the collected prescriptions. The high frequency CMs were mined by frequency analysis method, and the compatibility rule of prescription was analyzed by association rules. The rules package was called into the formula basket data by R software platform, and the Apriori algorithm was used to mine the data for association rules with Confidence, Support and Promotion as the criteria (CSBTS, 1997). Support value was the percentage of preconditions that were true and used to measure universality; Confidence value was the percentage of preconditions for which records and the conclusions were both true, mainly used for measuring accuracy; Promotion value was used to evaluate the degree to which the appearance of one item set increased the appearance of another (Zhan and Fu, 2016). The mined related knowledge was screened and visualized by the arulesViz package to construct the associated knowledge network (Hahsler et al., 2011).

2.2. Molecular docking

2.2.1. Screening of active components in CMs

The chemical composition of high-frequency CMs were obtained

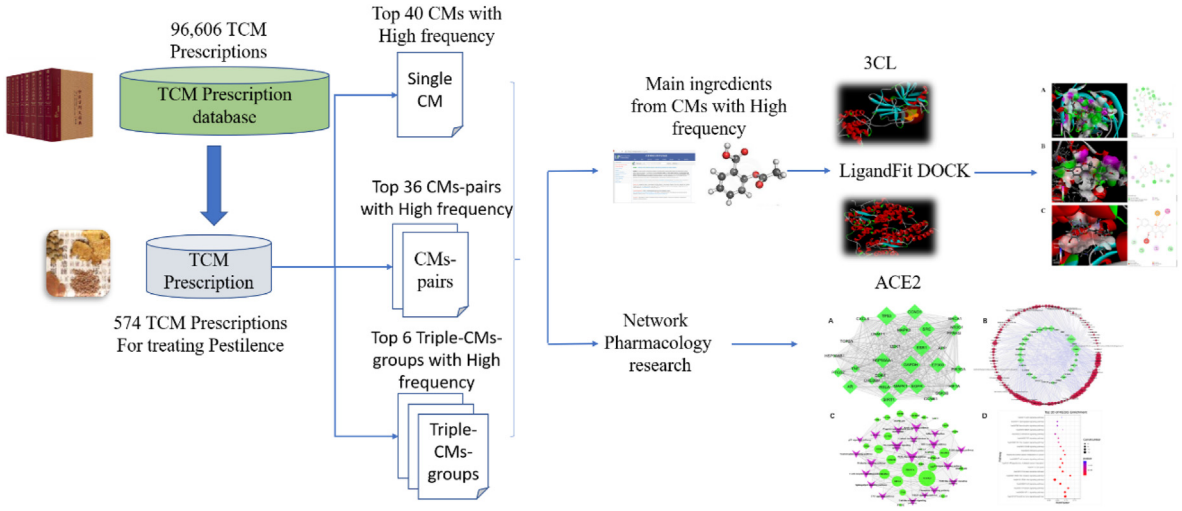

Fig. 1. The whole framework based on an integration strategy of screening system.

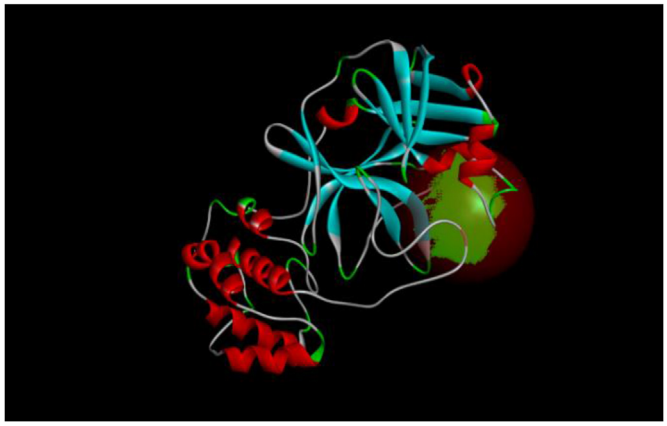

Fig. 2. High-resolution crystal structure of novel coronavirus target 3CL hydrolase (Mpro) (PDB 6LU7). The circle in the figure is the position of the active pocket.

from the TcmSP™ (Traditional Chinese Medicine System Pharmacology Database, http://tcmspw.com/tcmsp.php). Meanwhile, important pharmacology-related parameters of compounds were also obtained from TcmSP™, including drug-likeness (DL) and oral bioavailability (OB). The compounds with OB > 30% and DL > 0.18 were selected as candidate compounds for further analysis (Wang et al., 2017). Besides, some compounds with low OB or DL values were also selected for candidate compounds because of their excellent pharmacological activities or high contents (S. J. Yue et al., 2017). The sdf format of the main active ingredients' structures were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) as candidates for molecular docking.

2.2.2. Preparation of the target proteins and the active site

The high-resolution crystal structure of COVID-19 3CL hydrolase (Mpro) was obtained from PDB (PDB_ID: 6LU7) (Jin et al., 2015) (Fig. 2). The active site of the protein is centered on the active amino acid site of the original ligand in the crystal structure. The corresponding "active pocket" was constructed. The system searched for the "active pocket" near the active site, and finally −8.669631, 12.384467, and 67.029640 with a point count of 8538 were defined as active pocket.

The high-resolution crystal structure of angiotensin-converting enzyme 2 (ACE2) was obtained from PDB (PDB_ID:2AJF) (Fig. 2). The two active sites of the protein are centered on the active amino acid site of the original ligand in the crystal structure. The active site 1 of 15.262085, −17.780927, 57.786474 with the points count of 4198

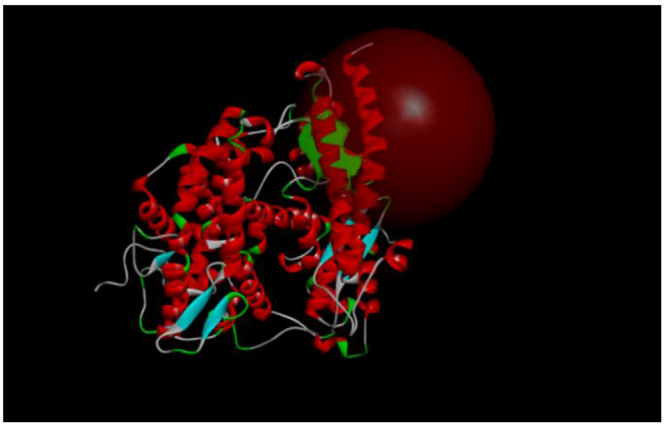

Fig. 3. High-resolution crystal structure of ACE2(PDB_ID:2AJF) site 1. The circle in the figure is the position of the active pocket.

(Fig. 3) and the active site 2 of 21.539614,7.755639,61.035558 with the points count of 4198 (Fig. 4) were constructed to dock.

2.2.3. Molecular docking method

Receptor-Ligand interaction module in Discovery Shannon, 2003) R2 were used to explore binding rates between the main ingredients in high frequency CMs and the key targets of SARS-COV-2. The LigandFit molecular docking parameter settings remained the default parameters.

In order to ensure the accuracy of the results, scoring was performed with seven scoring functions: DockScore, LigScore1, LigScore2, PLP1, PLP2, Jain and PMF. Then, consensus score was used to analysis of seven scoring functions selected for Ligandfit docking, which produces a single consensus score value for each ligand rather than for each posed to measure the result. The threshold was set at greater than four.

2.3. Potential action mechanisms of high-frequency CMs pairs

The Huangqin and Gancao CMs-pair were selected to explore their possible molecular mechanism. The chemical compositions were obtained according to "2.2.1 Screening of active components in CMs". We then predicted the potential targets using target prediction approach developed by Fu et al., (2017). Noteworthy, only the targets with reliability score greater than 0.9 of *Homo sapiens* were retained for further analysis. After, we established the Protein-Protein Interaction (PPI) network for the targets by using String Datasets (https://string-db.org/), and the PPI networks were further visualized by using Cytoscape (Version 3.5.0, available at http://www.cytoscape.org/) (Shannon, 2003).

DAVID, a database for annotation, visualization and integrated discovery to identify the functions, was used to perform GO enrichment analysis and KEGG pathway enrichment analyses for the potential targets (Huang et al., 2009). The *P*-value was calculated and further corrected by using the Benjamini-Hochberg method, and *P*-value < 0.05 was selected as the cutoff criterion. Subsequently, the compound-target network, and target-pathway network were constructed and visualized by using Cytoscape (Version 3.5.0).

3. Results and discussion

3.1. CMs prescriptions screening results

Chinese medicine prescriptions (fang ji in Chinese) is the main form of CMs application in clinical practice (Ren et al., 2019). *Dictionary of Traditional Chinese Medicine Prescriptions* is the summary of research achievement in CMs prescriptions, which contains more than 1800 kinds of CMs and 90, 000 prescriptions in related literatures (Peng, 1996). In our study, the prescriptions for treatment of pestilence or epidemic diseases were mined from *Dictionary of Traditional Chinese Medicine Prescriptions*. 574 prescriptions were selected for the treatment of "Wenbing", "Wenyi", "Yibing" or "Shiyi". The age distribution of prescriptions showed that the use of CMs to prevent epidemics could be traced back to Jin dynasty (Fig. 5). The selected prescriptions were mainly distributed in the Song, Ming and Qing dynasties, especially the Qing dynasty with 325 kinds of prescriptions. It was estimated that it may be due to the academic development of Seasonal Febrile Disease after the Ming dynasty. Dr. Wu Youke from Ming dynasty proposed the etiological theory of "liqi" for the first time, which mean the evil epidemic pathogenic factors in *Wen Yi Lun* (Wu, 1991). Wu emphasized that Wen diseases (pestilence) was totally different from febrile disease and clearly pointed out that *"The wenyi was a disease, not feng(wind), han(cold), shu(heat) or shi(damp), but a strange feeling between heaven and earth"*. In addition, Wu established the thinking mode of syndrome differentiation and created the effective prescription named "Dayuanyin" for treating pestilence diseases. While during the formation of Seasonal Febrile Disease school in the Qing Dynasty, febrile pathologists such as Ye Gui, Wu Jiao, Wang Shixiong and Xue Shengbai not

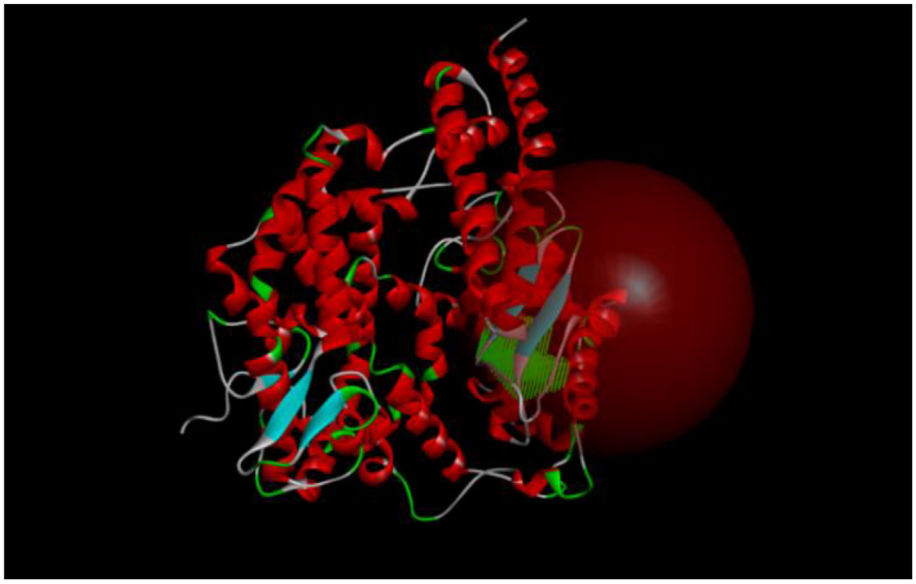

Fig. 4. High-resolution crystal structure of ACE2(PDB_ID:2AJF) site 2. The circle in the figure is the position of the active pocket.

only put forward the academic opinions of Wei Qi Ying Xue from *Wen Bing Lun* (Yue et al., 2017), Differentiation of Triple Energizer from *Wen Bing Tiao Bian* (Wu, 1972). Abundant publishes and excellent prescriptions of Wen diseases were also created in that times.

3.2. High frequency CMs from prescriptions

In order to screen high frequency CMs, we counted the frequency of the herbs used in screened CMs formulae by frequency analysis method. The results showed that 40 kinds of CMs with the highest frequency from above prescriptions (Table 1, Table 2). Glycyrrhizae Radix Et Rhizoma (Gancao; *Glycyrrhiza uralensis* Fisch, Rhizome) was the most frequency used in half of the CM prescriptions, followed by Scutellariae Radix (HuangQin; *Scutellaria baicalensis* Georgi, root) and Rhei Radix Et Rhizome (Dahuang; *Rheum palmatum* L., *Rheum tanguticum* Maxim. ex Balf., or *Rheum officinale* Baill., Rhizome), both of them were used more than 100 times. Most of these high frequency CMs were kinds of cold and heat-clearing CMs, which corresponding to the classic Warm disease's symptoms of syndrome differentiation of COVID-19. Among them, *HuangQin* (Scutellariae Radix) could relive lung heat corresponding to the high fever syndrome type of COVID-19, and *Dahuang* (Rhei Radix Et Rhizome) discharging damp-heat of large intestine, corresponding to diarrhea syndrome of some patients of COVID-19. At the same time, there were also some supplementing Qi and nourishing Yin CMs, such as Paeoniae Radix Alba (Baishao; *Paeonia lactiflora* Pall., root), Angelicae Sinensis Radix (Danggui; *Angelica sinensis* (Oliv.) Diels, root), Rehmanniae Radix (Shengdi; *Rehmannia glutinosa* (Gaertn.) DC., root) and Ginseng Radix Et Rhizoma (Renshen; *Panax ginseng* C.A.Mey., Rhizome), and expectorant CMs, such as Citri Reticulatae Pericarpium (Chenpi; *Citrus reticulata* Blanco, fruit) and Platycodonis Radix (Jiegeng; *Platycodon grandiflorus* (Jacq.) A.DC., root). The above results indicated the principle of Traditional Chinese Medicine with strengthening the body resistance to eliminate pathogenic factors and treating both symptom and root au.

3.3. High frequency CMs-pair and triple-CMs groups in prescriptions

CMs-pair and triple-CMs groups are the basic forms of compatibility of CMs. There were 36 CMs-pair with a frequency of more than 5% from above prescriptions (Table 3). *Gancao* (Glycyrrhizae Radix Et Rhizoma) was most commonly used in high frequency CMs-pair. Most frequently used CMs-pair was *Huangqin* (Scutellariae Radix) and *Gancao* (Glycyrrhizae Radix Et Rhizoma) pair, which was the sovereign drug of Gancao Huangqin Tang in the *Si Sheng Xin Yuan* (Huang, 2019). Huangqin is a frequently used CMs, and have the effects of clearing heat and depriving the evil wetness, heat-clearing and detoxicating, while *Gancao* (Glycyrrhizae Radix Et Rhizoma) have the effects of tonifying the middle body and supplementing Qi. *Huangqin* (Scutellariae Radix) and *Gancao* (Glycyrrhizae Radix Et Rhizoma) CMs-pair could be used for treating heat syndrome caused by SARS-COV-2.

As for triple-CMs-groups, Chuanxiong Rhizoma (Chuanxiong; *Ligusticum chuanxiong* Hort.), Notopterygii Rhizoma Et Radix (Qianghuo; *Notopterygium incisum* K.C.Ting ex H.T.Chang, *Notopterygium franchetii* H.Boissieu, root) and *Gancao* (Glycyrrhizae Radix Et Rhizoma) group was used most commonly in the prescription, followed by Bupleuri Radix (Chaihu; *Bupleurum chinense* DC., *Bupleurum*

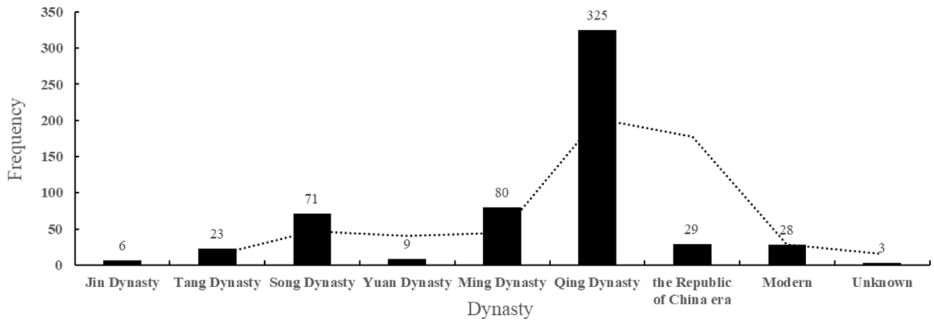

Fig. 5. Distribution times of 574 prescriptions for treatment of pestilence or epidemic diseases.

Table 1
The Names of High frequency CMs.

No.	Chinese Name	Latin name[a]	Source species	Parts used
1	Gancao	Glycyrrhizae Radix Et Rhizoma	*Glycyrrhiza uralensis* Fisch *Glycyrrhiza inflata* Batalin *Glycyrrhiza glabra* L.	Rhizome
2	Huangqin	Scutellariae Radix	*Scutellaria baicalensis* Georgi	Root
3	Dahuang	Rhei Radix Et Rhizome	*Rheum palmatum* L. *Rheum tanguticum* Maxim. ex Balf. *Rheum officinale* Baill.	Rhizome
4	Baishao	Paeoniae Radix Alba	*Paeonia lactiflora* Pall.	Root
5	Chenpi	Citri Reticulatae Pericarpium	*Citrus reticulata* Blanco	Fruit
6	Chaihu	Bupleuri Radix	*Bupleurum chinense* DC. *Bupleurum scorzonerifolium* Willd.	Root
7	Jiegeng	Platycodonis Radix	*Platycodon grandiflorus* (Jacq.) A.DC.	Root
8	Cangzhu	Atractylodes Rhizoma	*Atractylodes lancea* (Thunb.) DC. *Atractylodes chinensis* (DC.) Koidz.	Rhizome
9	Danggui	Angelicae Sinensis Radix	*Angelica sinensis* (Oliv.) Diels	Root
10	Shengdi	Rehmanniae Radix	*Rehmannia glutinosa* (Gaertn.) DC.	Root
11	Shigao	Gypsum Fibrosum	Gypsum	
12	Gegen	Puerariae Lobatae Radix	*Pueraria lobata* (Willd.) Ohwi	Root
13	Houpu	Magnolia Officinalis Cortex	*Magnolia officinalis* Rehder & E.H.Wilson *Magnolia officinalis* var. biloba Rehder & E.H.Wilson	Bark
14	Chuanxiong	Chuanxiong Rhizoma	*Ligusticum chuanxiong* Hort.	Root
15	Fangfeng	Saposhnikoviae Radix	*Saposhnikovia divaricata* (Turcz.) Schischk.	Root
16	Shexiang	Moschus	*Moschus berezovskii* Flerov. *Moschus sifanicus* Przewalski *Moschus moschiferus* Linnaeus	Musk bag
17	Huanglian	Coptidis Rhizoma	*Coptis chinensis* Franch. *Coptis deltoidea* C.Y.Cheng et Hsiao *Coptis teeta* Wall.	Rhizome
18	Qianghuo	Notopterygii Rhizoma Et Radix	*Notopterygium incisum* K.C.Ting ex H.T.Chang *Notopterygium franchetii* H.Boissieu	Rhizome
19	Xuanshen	Scrophulariae Radix	*Scrophularia ningpoensis* Hemsl.	Root
20	Baizhi	Angelicae Dahuricae Radix	*Angelica dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav.	Root
21	Renshen	Ginseng Radix Et Rhizoma	*Panax ginseng* C.A.Mey.	Root
22	Xionghuang	Realgar	Realgar	
23	Fuling	Poria	*Poria cocos*(Schw.)Wolf	Sclerotium
24	Zhiqiao	Aurantii Fructus	*Citrus* × *aurantium* L.	Fruit
25	Maidong	Ophiopogonis Radix	*Ophiopogon japonicus* (Thunb.) Ker Gawl.	Root
26	Jiangcan	Bombyxbatryticatus	*Beauveriaassiana*(Bals.)Vuillant	Silkworm body
27	Lianqiao	Forsythiae Fructus	*Forsythia suspensa* (Thunb.) Vahl	Fruit
28	Zhimu	Anemarrhenae Rhizoma	*Anemarrhena asphodeloides* Bunge	Rhizome
29	Banxia	Pinelliae Rhizoma	*Pinellia ternata* (Thunb.) Makino	Rhizome
30	Bohe	Menthae Haplocalycis Herba	*Mentha haplocalyx* Briq.	Stem
31	Zhusha	Cinnabaris	Cinnabar	
32	Shengma	Cimicifugae Rhizoma	*Cimicifuga heracleifolia* Kom. *Cimicifuga dahurica* (Turcz.) Maxim. *Cimicifuga foetida* L.	Rhizome
33	Mahuang	Ephedra Herba	*Ephedra sinica* Stapf *Ephedra intermedia* Schrenk & C.A.Mey. *Ephedra equisetina* Bunge	Stem
34	Zhizi	Gardeniae Fructus	*Gardenia jasminoides* J.Ellis	Fruit
35	Chantui	Cicadae Periostracum	*Cryptotympana pustulata* Fabricius	
36	Tianhuafen	Trichosanthis Radix	*Trichosanthes kirilowii* Maxim. *Trichosanthes rosthornii* Harms	Root
37	Shengjiang	Zingiber Rhizoma Recens	*Zingiber officinale* Roscoe	Rhizome
38	Xixin	Asari Radix Et Rhizoma	*Asarum sieboldii* Miq. *Asarum heterotropoides* F.Schmidt	Rhizome
39	Huashi	Talcum	Talcum	
40	Huoxiang	Pogostemonis Herba	Pogostemon amaranthoides *Benth.*	Stem

[a] The Latin names by Chinese Pharmacopoeia (2015 Edition).

scorzonerifolium Willd., root), *Huangqin* (Scutellariae Radix) and *Gancao* (Glycyrrhizae Radix Et Rhizoma) groups (Table 4). The CMs-pair of *Qianghuo* (Notopterygii Rhizoma Et Radix) and *Chuanxiong* (Chuanxiong Rhizoma) had properties of enhancing in compatibility, which was used in Jiuwei Qianghuo Decoction and Da Qianghuo Decoction recorded in *Difficult to Know* (Wang, 1956). *Qianghuo* (Notopterygii Rhizoma Et Radix) belongs to Taiyang meridian which performed guiding role in medicine applying; While Chuanxiong was belonged to Jueyin meridian played the role as guiding medicine. The combination of *Qianghuo* (Notopterygii Rhizoma Et Radix) and *Chuanxiong* (Chuanxiong Rhizoma) showed better pharmacological effects in headache therapy originated from the Taiyang and/or Jueyin, and could be helpful for headache syndrome caused by SARS-COV-2.

3.4. Analysis of association rules and network of compatibility in CM prescriptions

Association rule mining is often used to find possible associations or connections between substance, and it has been applied to study the compatibility of CMs prescriptions (Zhang et al., 2020). We mined the association relationship (Supplementary Table S1) and matrix analysis (Fig. 6) for the compatibility relationship of CMs and built the

Table 2
High frequency CMs from Prescriptions (top 40).

No.	*Pinyin* Name	Latin name	Frequency	Percentage, %
1	Gancao	Glycyrrhizae Radix Et Rhizoma	296	51.57
2	Huangqin	Scutellariae Radix	123	21.43
3	Dahuang	Rhei Radix Et Rhizome	103	17.94
4	Baishao	Paeoniae Radix Alba	97	16.9
5	Chenpi	Citri Reticulatae Pericarpium	91	15.85
6	Chaihu	Bupleuri Radix	74	12.89
7	Jiegeng	Platycodonis Radix	74	12.89
8	Cangzhu	Atractylodes Rhizoma	69	12.02
9	Danggui	Angelicae Sinensis Radix	69	12.02
10	Shengdi	Rehmanniae Radix	69	12.02
11	Shigao	Gypsum Fibrosum	69	12.02
12	Gegen	Puerariae Lobatae Radix	66	11.5
13	Houpu	Magnolia Officinalis Cortex	65	11.32
14	Chuanxiong	Chuanxiong Rhizoma	63	10.98
15	Fangfeng	Saposhnikoviae Radix	62	10.8
16	Shexiang	Moschus	62	10.8
17	Huanglian	Coptidis Rhizoma	61	10.63
18	Qianghuo	Notopterygii Rhizoma Et Radix	60	10.45
19	Xuanshen	Scrophulariae Radix	58	10.1
20	Baizhi	Angelicae Dahuricae Radix	57	9.93
21	Renshen	Ginseng Radix Et Rhizoma	55	9.58
22	Xionghuang	Realgar	55	9.58
23	Fuling	Poria	54	9.41
24	Zhiqiao	Aurantii Fructus	54	9.41
25	Maidong	Ophiopogonis Radix	53	9.23
26	Jiangcan	Bombyxbatryticatus	52	9.06
27	Lianqiao	Forsythiae Fructus	52	9.06
28	Zhimu	Anemarrhenae Rhizoma	52	9.06
29	Banxia	Pinelliae Rhizoma	51	8.89
30	Bohe	Menthae Haplocalycis Herba	51	8.89
31	Zhusha	Cinnabaris	51	8.89
32	Shengma	Cimicifugae Rhizoma	48	8.36
33	Mahuang	Ephedra Herba	46	8.01
34	Zhizi	Gardeniae Fructus	44	7.67
35	Chantui	Cicadae Periostracum	41	7.14
36	Tianhuafen	Trichosanthis Radix	41	7.14
37	Shengjiang	Zingiber Rhizoma Recens	40	6.97
38	Xixin	Asari Radix Et Rhizoma	40	6.97
39	Huashi	Talcum	38	6.62
40	Huoxiang	Pogostemonis Herba	38	6.62

association knowledge network of CMs (Fig. 6). From these results, *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) were key medicines of these prescriptions for treatment of pestilence or epidemic diseases. Most of the CM prescriptions were designed basing on these two CMs.

3.5. LigandFit DOCK results of ingredients from high frequency CMs with SARS-COV-2 targets

The chemical composition of high frequency CMs were obtained from the Traditional Chinese Medicine System Pharmacology Database. There are 40 kinds of high-frequency CMs, five of which are not included in the database. Although a single CM usually contains a large number ingredient, only those with desirable pharmacodynamic and pharmacokinetic properties are key compounds for their therapeutic effects. In our current study, OB and DL were employed to screen candidate compounds from these high frequency CMs. In total, 35 high-frequency CMs includes 431 chemicals, which were molecularly docked with the SARS-COV-2 targets 3CL hydrolase and angiotensin converting enzyme 2 (ACE2) using LigandFit.

Consensus scoring, the combination of multiple scoring functions, is easier to find false positive than a single scoring function. The higher the Consensus scoring, the higher the binding rate of the molecule to the target. The score is greater than 4, indicating a better docking result. In our study, compounds with scoring values greater than 4 were screened for analysis. Therefore, 66 compounds were screened, of which 27 were docked with the 3CL hydrolase target and 48 were docked with the ACE2 target. The screened compounds were distributed in 27 kinds of CMs, among which *Gancao* (Glycyrrhizae Radix Et Rhizoma), *HuangQin* (Scutellariae Radix), *Dahuang* (Rhei Radix Et Rhizome) and *Chaihu* (Bupleuri Radix) contain more potential compounds (Fig. 7, Supplementary Table S2). The results of molecular docking were consistent with the frequency results of high frequency CMs.

3C-like protease ($3CL^{Pro}$) play an important role in the replication of the virus, which is considered to be an attractive target for drug development (Li et al., 2020). Acetoside (Consensus scoring = 7) has the strongest binding activity to 3CL hydrolase, which comes from *Shengdi* (Rehmanniae Radix). In Acetoside, hydrogen bonds were formed between phenolic hydroxyl groups and residues THR and PHE, and hydrophobic interaction was formed between benzene ring and target protein GLU (Fig. 8A). In addition, various components in high frequency CMs, such as *Gancao* (Glycyrrhizae Radix Et Rhizoma), *Dahuang* (Rhei Radix Et Rhizome) and *Chaihu* (Bupleuri Radix), also have potential anti-activity on 3CL protein (Supplementary Table S2).

According to the two binding regions grid3 and grid4 between ACE2 and viral protein conformation (Niu et al., 2020), components which may block the binding of the two proteins were screened. Glyasperin F in *Gancao* (Glycyrrhizae Radix Et Rhizoma) had the strongest binding to site ACE 1(Consensus scoring = 6), and hydrogen bond and σ-p hyperconjugated system was formed between its phenolic hydroxyl group forms and target protein residue LEU (Fig. 8B). Isorhamnetin in *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *Chaihu* (Bupleuri Radix) has the strongest binding ability to ACE site 2 (Consensus scoring = 6), which mainly formed hydrophobic interaction between benzene ring and residue target protein residue PRO (Fig. 8C). Besides, various ingredient in CMs, such as *HuangQin* (Scutellariae Radix), *Chaihu* (Bupleuri Radix) and *Zhimu*(Anemarrhenae Rhizoma), could combined with ACE2 protein.

3.6. Systemic pharmacological analysis of high-frequency CMs pairs

According to the frequency analysis, association rule analysis and molecular docking results of high-frequency CMs, *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) were the key medicines pairs of these prescriptions for treatment of COVID-19. A systemic pharmacology model based on chemical, pharmacokinetic and pharmacological data was constructed to explore the molecular mechanisms.

In the present work, the number of 85 and 34 kinds of active compounds were selected from *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix), respectively. The detailed information about those molecules were provided in Supplementary Table S3. An integrated *in silico* approach was introduced to identify the target proteins for the active compounds of CMs (Fu et al., 2017). We totally obtained 286 potential therapeutic targets for 119 kinds of candidate compounds from *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix). Then, we constructed a PPI (335 nodes and 4237edges) network for the putative targets of the compounds. Based on the average values for degree and distance of 21 and 2.3, respectively, we have identified 30 significant targets from *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) (Fig. 9A, Supplementary Table S4). In order to directly represent the interpretation of the complex relationships between active compounds and their targets, C-T network was constructed (Fig. 9B). Amongst them, those ones with high interconnection degrees were responsible for the high interconnectedness of the C-T network, especially Quercetin (degree = 10), 5,7,2′,6′-Tetrahydroxyflavone (degree = 12), Kaempferol (degree = 12), 4′-Hydroxywogonin (degree = 11), Ganhuangenin (degree = 11), Baicalein (degree = 9), Gancaonin O (degree = 8), and Norwogonin (degree = 8). As shown in the C-T network (Fig. 9B), a compound regulated multiple targets, while multiple compounds possibly regulate the same target.

Table 3
High frequency CMs-pair in prescriptions(percentage > 5%).

No.	CMs-pair	Frequency	Percentage, %
1	*Huangqin* (Scutellariae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	89	15.48
2	*Baishao* (Paeoniae Radix Alba) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	80	13.91
3	*Chenpi* (Citri Reticulatae Pericarpium) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	74	12.87
4	*Chaihu* (Bupleuri Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	59	10.26
5	*Gegen* (Puerariae Lobatae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	56	9.74
6	*Qianghuo* (Notopterygii Rhizoma Et Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	56	9.74
7	*Chuanxiong* (Chuanxiong Rhizoma) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	55	9.57
8	*Jiegeng* (Platycodonis Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	55	9.57
9	*Fangfeng* (Saposhnikoviae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	51	8.87
10	*Zhiqiao* (Aurantii Fructus) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	48	8.35
11	*Danggui* (Angelicae Sinensis Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	48	8.35
12	*Houpu* (Magnolia Officinalis Cortex) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	47	8.17
13	*Cangshu* (Atractylodes Rhizoma) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	44	7.65
14	Baizhi (Angelicae Dahuricae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	43	7.48
15	*Shigao* (Gypsum Fibrosum) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	42	7.3
16	*Fuling* (Poria) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	42	7.3
17	*Huanglian* (Coptidis Rhizoma) & *Huangqin* (Scutellariae Radix)	41	7.13
18	*Renshen* (Ginseng Radix Et Rhizoma) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	40	6.96
19	*Baizhi* (Angelicae Dahuricae Radix) & *Chuanxiong* (Chuanxiong Rhizoma)	39	6.78
20	*Zhimu* (Anemarrhenae Rhizoma) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	38	6.61
21	*Xuanshen* (Scrophulariae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	38	6.61
22	*Bohe* (Menthae Haplocalycis Herba) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	38	6.61
23	*Chuanxiong* (Chuanxiong Rhizoma) & *Qianghuo* (Notopterygii Rhizoma Et Radix)	38	6.61
24	*Xionghuang* (Realgar) & *Shexiang* (Zingiber Rhizoma Recens)	37	6.43
25	*Zhizi* (Gardeniae Fructus) & *Huangqin* (Scutellariae Radix)	35	6.09
26	*Lianqiao*(Forsythiae Fructus) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	35	6.09
27	*Banxia* (Pinelliae Rhizoma) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	35	6.09
28	*Chantui* (Cicadae Periostracum) & *Jiangcan* (Bombyxbatryticatus)	33	5.74
29	*Xionghuang* (Realgar) & *Zhusha* (Cinnabaris)	33	5.74
30	*Zhusha* (Cinnabaris) & *Shexiang* (Moschus)	32	5.57
31	*Shengma* (Cimicifugae Rhizoma) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	32	5.57
32	*Mahuang* (Ephedra Herba) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	31	5.39
33	*Tianhuafen* (Trichosanthis Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	31	5.39
34	*Bohe* (Menthae Haplocalycis Herba) & *Jiegeng* (Platycodonis Radix)	30	5.22
35	*Bingpian* (Borneolum) & *Shexiang* (Moschus)	29	5.04
36	*Huoxiang* (Pogostemonis Herba) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	29	5.04

Table 4
High frequency Triple-CMs-group in prescriptions(percentage > 5%).

No.	Triple-CMs-group	Frequency	Percentage, %
1	*Chuanxiong* (Chuanxiong Rhizoma) & *Qianghuo* (Notopterygii Rhizoma Et Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	38	6.61
2	*Chaihu* (Bupleuri Radix) & *Huangqin* (Scutellariae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	37	6.43
3	*Baizhi* (Angelicae Dahuricae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma) & *Chuanxiong* (Chuanxiong Rhizoma)	32	5.57
4	*Fangfeng* (Saposhnikoviae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma) & *Qianghuo* (Notopterygii Rhizoma Et Radix)	32	5.57
5	*Chaihu* (Bupleuri Radix) & *Qianghuo* (Notopterygii Rhizoma Et Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	32	5.57
6	*Chuanxiong* (Chuanxiong Rhizoma) & *Fangfeng* (Saposhnikoviae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	29	5.04

The significant targets interacting with the active ingredients were mapped onto the KEGG pathways and the T-P network was generated as shown in Fig. 9C. Among the results of KEGG pathway enrichment, we selected the pathways in the basic biological processes of metabolism, genetic information processing, environmental information processing, cellular processes and organismal system. There are 20 target-enriched pathways (Table 5), which act on the immune system, inflammation, cellular processes, and endocrine system, respectively. Thus, we postulated that *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) CM pair exerts therapeutic effects on multiple targets and pathways of the human body through hits complex active component.

Under the guidance of the theory of “treating non-disease” in TCM, CMs and CM prescriptions play a multi-effect synergistic effect, which makes them have more significant advantages in anti-COVID-19 (Luo et al., 2020). The classical CM prescriptions condense the experience in fighting against epidemics diseases for thousands of years. Its successful effects have been preliminarily confirmed in clinical studies when applied to SARS and H1N1 influenza epidemics (Lau et al., 2005,Liu et al., 2013). Meanwhile, Qing-Fei-Pai-Du decoction is a CM prescription recommended by the National Health Commission of China for the treatment of COVID-19, which was optimized by combination of a number of classical prescriptions from *Treatise on Febrile and Miscellaneous* (Han dynasty) (Zhong, 1963). Thus, the experiences and effective prescriptions of CM in treating and preventing viral infection diseases for thousands of years is worthwhile to explore and are of great significance and reasonable.

We excavated 574 CM prescriptions for treating epidemic diseases through 96, 606 prescription. Among them, 40 kinds of high frequency CMs, 36 high-frequency CMs-pairs and 6 kinds of high-frequency triple-CM-groups were mined by frequency analysis method were used to analyze the collected prescriptions. Among the commonly used CMs beneficial for antiviral, *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) were given high priority CM pairs for selection used in CM prescriptions against pestilence by mining the compatibility rules of prescriptions with association rules analyses. The molecular docking results implied that 66 compounds in 26 kinds of CMs probably show a potential anti-SARS-COV-2 activity by binding

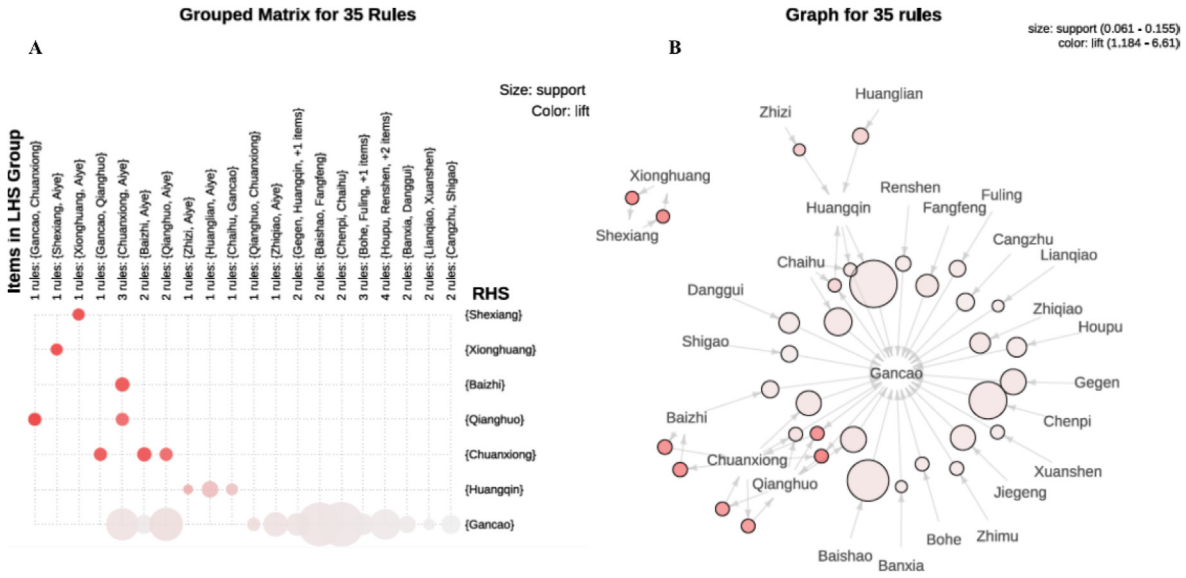

Fig. 6. CMs incidence matrix of prescription (A) and CMs association network of prescription (B).

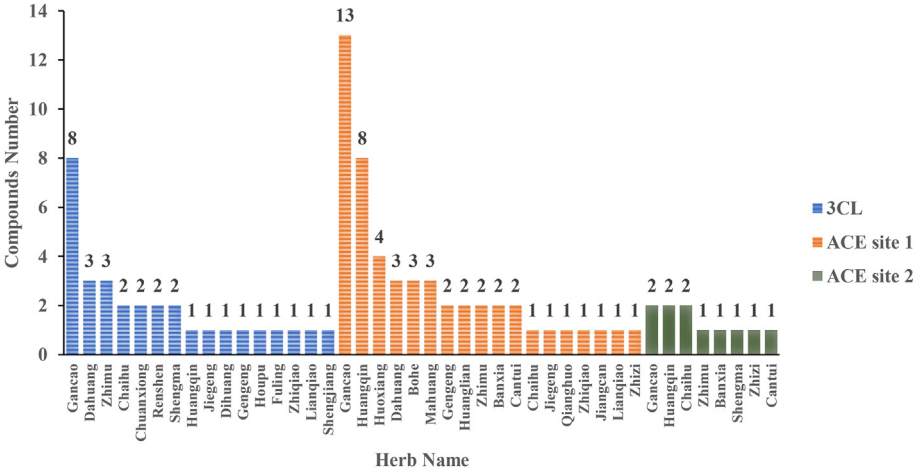

Fig. 7. High Frequency CMs with active components distribution.

with the ACE2 and 3CL hydrolase. It's worth noting that *Gancao* (Glycyrrhizae Radix Et Rhizoma) contained the most potential compounds of 20, followed by *HuangQin* (Scutellariae Radix). Meanwhile, based on network-based computational methods, an integrated system pharmacology approach was used to predict targets, construct networks, and explore the molecular action of high-frequency *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) (GH) CMs-pair. In present study, 85 and 34 kinds of active ingredients with favorable bioactivities and contents were selected from *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) by ADME filtering, providing some foundational clues for thorough investigation on this CMs-pair. By analyzing the network topology of targets, 30 kinds of important targets were identified. By using network systematic analysis, GH CMs-pair could regulate the proteins related to immune system, inflammation, cellular processes, and endocrine system. COVID-19 leads to a strong immune response and inflammatory storm, in which a large number of cytokines are activated. GH CMs-pair may regulate the immune-related pathway Toll-like receptor signaling pathway, T-cell and B-cell receptor signaling pathway, as well as cytokine action related pathways such as TNF signaling pathway, NF-κB signaling pathway and PI3K-Akt signaling pathway signaling pathway to inhibit the activated cytokines, relieve the excessive immune response and eliminate inflammation. From perspective of molecular network, GH CMs-pair exerted overall regulation through multi-ingredient and multi-target synergistic effect.

4. Conclusion

In conclusion, based on experience of ancient prescription and modern pharmacy research methods, 40 kinds of high frequency CMs, 36 high-frequency CMs-pair and 6 kinds of high-frequency triple-CMs-group were excavated. In addition, the molecular mechanism of the selected key CMs drug pair was preliminarily discussed. *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) CMs-pair with highest frequency show a potential anti-SARS-COV-2 activity by binding with the ACE2 and 3CL hydrolase and regulate the target related to immune system, inflammation, cellular processes, and endocrine system. Our results provide referenced candidate compatibility of CM and active ingredients against SARS-COV-2. The results fully reflected the synergistic mechanism of multi-components and multi-targets of CMs. In view of the limitations of virtual screening results, further experiments *in vivo* and *in vitro* are needed to verify the results of this study in the later stage, so as to provide experimental basis for the research and development of antiviral natural drugs.

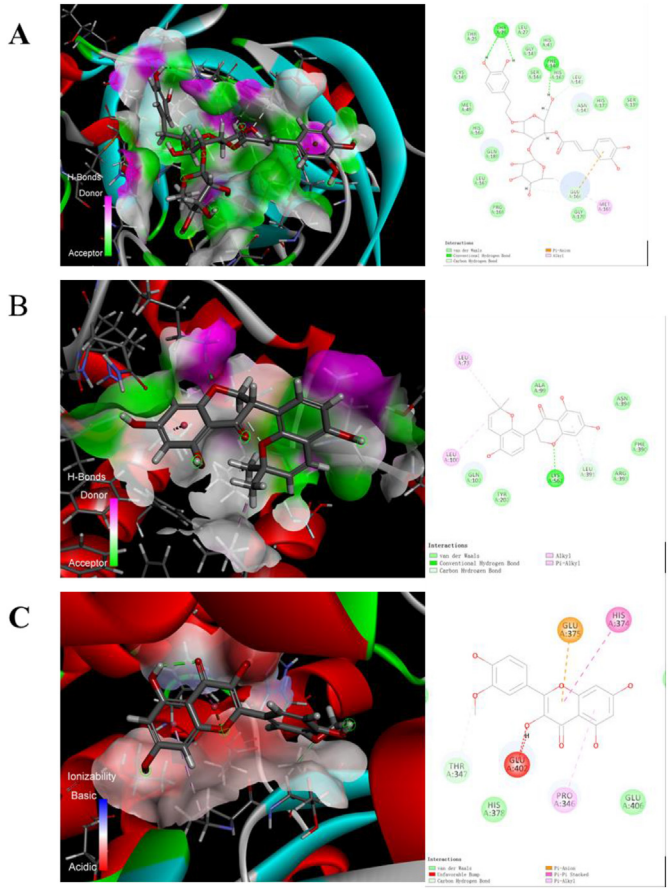

Fig. 8. Molecular docking patterns of candidate compounds with SARS-COV-2 targets. A: Acetoside with 3CL; B: Glyasperin F with ACE2 stie 1; C: Isorhamnetin with ACE2 site 2.

Table 5
KEGG pathways regulated by *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) CMs-pair target.

Pathway classification	Pathway	Count	*P*-Value
Immune system	NOD-like receptor signaling pathway	7	7.26E-08
	T cell receptor signaling pathway	7	2.36E-06
	Toll-like receptor signaling pathway	6	5.86E-05
Inflammation	PI3K-Akt signaling pathway	12	4.29E-08
	TNF signaling pathway	6	6.13E-05
	Chemokine signaling pathway	7	8.27E-05
Angiogenesis	VEGF signaling pathway	5	9.77E-05
Nervous	Neurotrophin signaling pathway	6	0.000106
	ErbB signaling pathway	6	2.25E-05
Cellular processes	Adherens junction	6	8.3E-06
	Cell cycle	8	4.51E-07
	Sphingolipid signaling pathway	6	0.000106
	p53 signaling pathway	5	0.000141
	HIF-1 signaling pathway	9	2.65E-09
	FoxO signaling pathway	9	3.77E-08
	Central carbon metabolism in cancer	6	4.96E-06
Endocrine system	Thyroid hormone signaling pathway	11	1.17E-11
	Estrogen signaling pathway	8	9.55E-08
	Progesterone-mediated oocyte maturation	7	1.04E-06
	Prolactin signaling pathway	8	9.2E-09

Notes

The authors declare no competing financial interest.

Author contributions

Xian-Jun Fu, Zhen-Guo Wang, Xin-Hua Jia, Tao Song, Wu-Yi Zhou and Yan-Gang Zhao conceived and designed the experiments; Xia Ren, Xin-Xin Shao, Xiu-Xue Li, Yang Li, Xiao-Long Wang, Zhen-Yang Li,Yue Zhong performed the experiments; Xia Ren wrote the original draft; Peng Wang, Qing-Hua Cui; Pei-Ju Qin, Xue-Bo Li, Feng-Cong Zhang edited and reviewed the paper. All authors have read, revised and approved the final manuscript.

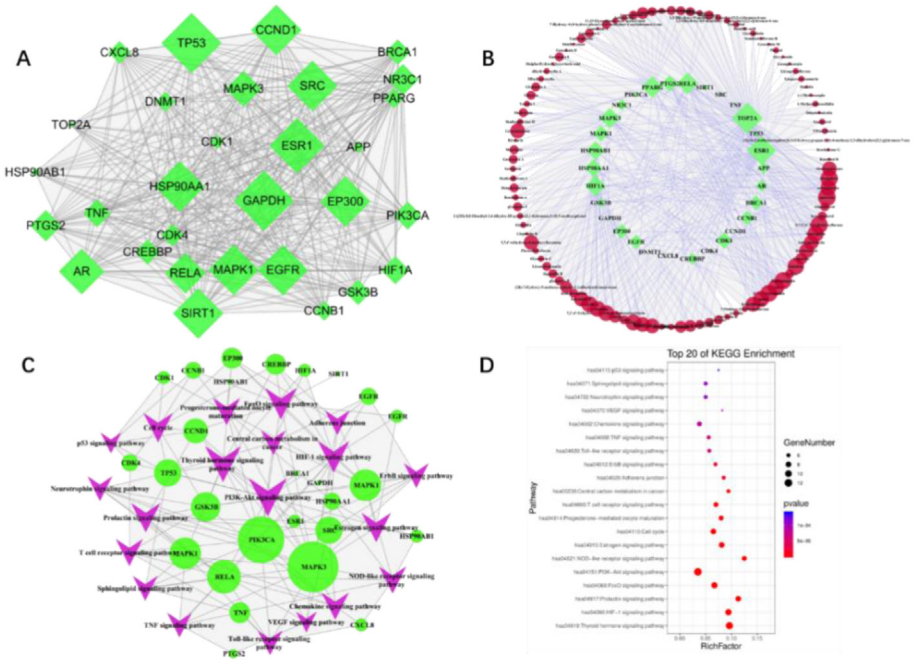

Fig. 9. Potential action mechanisms of *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) CMs-pair. A: PPI network of candidate Gancao-Huangqin CMs-pair targets; B: Construction of the Gancao-Huangqin CMs-pair compound-target network. The nodes representing candidate compounds are shown as red, and the targets are indicated as green; C:Construction of the Gancao-Huangqin CMs-pair target-pathway network; D: Pathway enrichment analysis of candidate targets. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2020.112932.

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