A network pharmacological study to investigate the combination of LHQW-XYS in the treatment of COVID-19 olfactory impairment-associated

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Abstract: To explore the target of action of LHQW-XYS on the main components of COVID-19 olfactory impairment by using network pharmacological methods and try to reveal its mechanism of action in the treatment related to COVID-19 induced olfactory impairment, we used the TCMSP platform to obtain potential active ingredients through oral utilization and drug-like properties screening; the Swiss TargetPrediction platform to predict the targets of the active ingredients and construct a drug-ingredient-target network, and then obtained the gene targets of COVID-19 olfactory injury through GeneCards, OMIM, and TTD platforms to intersect the drug targets and disease genes to obtain common targets. The drug targets and disease genes were intersected to obtain common targets. STRING and Cytoscape 3.8.2 software were used to construct the target-disease gene PPI network, screen the key targets and core gene clusters, and analyze the key targets by GO and KEGG enrichment analyses with the help of the Metascape platform, and then map the screened core active ingredients and their targets into the pathway to construct the core active ingredients-targets-pathway network. The core active ingredient-target-pathway network was constructed, and finally, molecular docking was carried out. The results showed that there were 4669 potential targets, 5609 disease targets, and 17 drug-disease cross-targets for the active ingredients of LHQW-XYS. The GO and KEGG enrichment analyses indicated that the mechanism of LHQW-XYS in the treatment of olfactory impairment in COVID-19 may be due to the regulation of related signaling pathways, such as Serotonergic synapse and Regulation of lipolysis in adipocytes. Molecular docking showed that six active components (quercetin, luteolin, kaempferol, 7-methoxy-2-methylisoflavone, wogonin, medicarpin) and two key genes (PTGS2, PPARG) had good binding properties. In the end, we conclude that LHQW-XYS may act on Serotonergic synapse and Regulation of lipolysis in adipocyte pathways to achieve anti-COVID-19 olfactory impairment-associated effects.

Keywords: LHQWXYS; COVID-19 olfactory impairment-associate; Network pharmacology; Molecular docking

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1. Introduction

Novel coronavirus pneumonia is an acute respiratory infection caused by the SARS-CoV-2 virus that poses a serious threat to the lives and health of people worldwide[1]. Many patients begin to experience varying degrees of olfactory disturbance (hyposmia/absence of smell) early in the course of neocoronary pneumonia[2–8]. 11.8%–35.5% of patients with viral infection develop olfactory disturbances without other clinical symptoms[9,10]. Recent epidemiological studies have shown that the prevalence of olfactory impairment in patients with neocoronavirus pneumonia exceeds 80%[9]. Current evidence suggests that the cause of hyposmia or loss of smell in patients after neocoronavirus pneumonia may be related to the targeted destruction of olfactory epithelial support cells and stem cells by the virus and the development of obstructive inflammation in the olfactory fissure[11,12]. Sudden loss of smell will cause

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anxiety in patients, which is more detrimental to the recovery of the disease and seriously affects the quality of life of patients[13]. However, there is no specific drug treatment available, and Western medicine generally intervenes with treatment strategies such as corticosteroid sprays[14], nasal rinses with competitive ACE2-inhibiting drugs[15], and olfactory training[16], but the overall efficacy is poor.

Traditional Chinese medicine (TCM) has been instrumental in the treatment and control of Neoplastic pneumonia. Lianhua Qingwen capsule (LHQW) is one of the officially approved TCM preparations for the treatment of Neoplastic pneumonia, which has antiviral and immune modulating effects[17]. Xinyi San (XYS) was first published in the "Yan's Jisheng Formula" by Yan Yonghe in the Song Dynasty. In the book, it is written that Xinyi San can treat the symptom of "no smell or odor", and the prescription is "equal parts each of XinYiRen, XiXin (wash off the soil and leaves), GaoBen (remove roots and stems, etc.), ShengMa, ChuanXiong, MuTong (remove the knot), FangFeng (remove roots and stems, etc.), QiangHuo (remove roots and stems, etc.), licorice (honey-fried) and BaiZhi", which has been handed down to the present day and is widely used. The combination of Lianhua Qingwen capsule and Xinyi San (LHQW-XYS) is effective for hyposmia after neocoronavirus pneumonia, but it has not been studied enough. Network pharmacology, based on the theory of systems biology, uses network analysis of biological systems to select specific signaling nodes and construct a complex network between active ingredients-protein targets-signaling pathways to explore the mechanism of action of drugs. Therefore, this study mainly used network pharmacology to conduct a preliminary investigation on the main components, targets and mechanisms of action of LHQW-XYS in the treatment of hyposmia (disorder) after neocoronavirus pneumonia, in order to provide a reference for clinical treatment.

2. Methods and results

2.1. Screening of active compounds of LHQW-XYS

The active compounds of LHQW-XYS were screened using the traditional Chinese medicine systems pharmacology (TCMSP) database (https://tcmspw.com/tcmsp.php). The PubChem ID, molecular formula, and canonical simplified molecular input line entry specification (SMILES) of each component were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). TCMSP is a unique systemic pharmacology platform for Chinese herbal medicines that analyzes the relationships between drugs, targets, and diseases[18]. Oral bioavailability (OB) is an important pharmacokinetic parameter in drug absorption, distribution, metabolism, and excretion (ADME)[19]. Drug-like (DL) properties refers to the similarity of a compound to a known drug, and in drug development, DL studies are based on lead compounds, with DL molecules considered as high-quality lead compounds[20]. In this study, compounds with OB ≥ 30% and DL ≥ 0.18 were selected as potential active components. The target information of these active components was standardized by using the Uniprot database (https://www.uniprot.org/) with the species set as "homo sapiens".

2.2. Exploring of the COVID-19 olfactory impairment potential targets

We searched several databases, including GeneCards (https://www.genecards.org/), online Mendelian inheritance in man (OMIM) (https://www.omim.org/), NCBI (https://www.ncbi.nlm.nih.gov/)[21], and Drugbank (https://go.drugbank.com/), using the keyword "COVID-19" and "Olfactory impairment" to screen for the targets of LHQW-XYS combined with COVID-19 olfactory impairment active compounds. Only the targets with a score ≥ 10 were chosen from the GeneCards database.

After the removal of repeated targets, Venny 2.1.0 was used to screen the intersection of drug targets and disease targets to obtain the potential targets of LHQW-XYS in COVID-19 olfactory impairment treatment (core targets).

2.3. Network construction

Subsequently, we formed the "LHQW-XYS compound–COVID-19 olfactory impairment target" interaction network. The core targets were imported into Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; https://string-db.org/), and predicted protein-protein interactions (PPI) were obtained at a high confidence score > 0.9. The above results were imported into the Cytoscape 3.9.1 (https://www.cytoscape.org/) software to visualize the complex relationships between the LHQW-XYS active components and the potential COVID-19 olfactory impairment associated target genes[22], to construct a regulatory network of LHQW-XYS targets for COVID-19 olfactory impairment treatment. The layout tool was used to quantify the degree of each node, with the node size corresponding to the degree value of the node.

2.4. PPI analysis and core gene screening

The intersecting gene targets of LHQW-XYS and COVID-19 olfactory impairment were imported into the STRING protein interaction analysis platform, and the protein classification was set to "homo sapiens" with a maximum confidence level of ≥ 0.7, hiding the unlinked nodes in the network. Protein interaction network analysis was performed and the TSV file was downloaded. Cytoscape 3.9.1 software was imported to construct PPI network maps. The hub targets in the PPI network were screened using the cytoHubba plugin, with darker colored nodes representing higher scores[23]. The molecular complex detection (MCODE) plugin was used to discover closely linked regions in the PPI network[24]. The score value of a module reflects how dense the module is to the surrounding nodes, with higher scores indicating greater importance of the node.

2.5. GO and KEGG enrichment analyses

GO functional analysis is used to determine the function of the gene targets, including biological process, cellular component, and molecular function, while KEGG enrichment analysis determines the signal pathways enriched by the gene targets. The intersecting genes of the LHQW-XYS and COVID-19 olfactory impairment-associated targets were analyzed by GO and KEGG enrichment analyses, using R 3.6.3 software-related R packages, to determine their biological functions. According to the Fisher's test, $P < 0.05$ and $q < 0.05$ were considered statistically significant.

2.6. Molecular docking

2.6.1. Downloading structure files and energy minimization

Prior to running the docking program, we downloaded the 2D structures of the six active components from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and used ChemBioffice 2014 for energy minimization.

2.6.2. Screening for suitable protein receptors

Among the ligand receptors downloaded from RCSB PDB (https://www.rcsb.org/), 5F1A, 5IKR, 5IKV, 6FZG were chosen as suitable ligand receptors. Thereafter, Sybyl-X (https://sybyl.com/) was used to run the docking program.

2.6.3. Visualization of molecular docking results

LHQW-XYS compounds with a high number of targets were selected for molecular docking with the core

targets (PTGS2 and PPARG) in the PPI network map. The binding energy (Vina score) was used to evaluate the bonding strength between the docked molecules. The smaller the Vina score, the higher the affinity of the receptor and ligand and the more stable the binding of the compound to the target site. The binding energies of the four active components and the corresponding target proteins were < –5.0, indicating efficient docking and high binding activity[25]. Molecular docking simulations showed a stable point docking structure for the binding of small-molecule ligands and protein receptors.

2.6.4. Molecular docking

The top 10 target proteins in the PPI network were selected for molecular docking. The PDB format file of the 3D structure of the targets were downloaded from the RCSB PDB (https://www.rcsb.org/) database and the mol2 format file of the 3D structure of the core active components were downloaded from the TCMSP database. SYBYL-X 2.0 was used to run the RMSD program to choose the right PDB structure to use as the molecular docking ligand. Docking was performed using the active components of the LHQW-XYS and core target proteins using AutoDock Vina, after importing the files from PDB to AutoDockTools[26] for hydrogenation, dehydration, and other pretreatments. The docking results were visualized using PyMoL 2.6.0 software[27], and the binding activity was determined by evaluating the binding efficiency.

3. Results

3.1. Active components and potential targets of LHQW-XYS

A total of 4669 LHQW-XYS active components were screened for their potential targets by searching the TCMSP database at threshold values of OB ≥ 30% and DL ≥ 0.18 (Table 1).

3.2. COVID-19 olfactory impairment-associated gene targets

A total of 3432, 200, 119, and 5 genes were retrieved from the GeneCards, OMIM, NCBI, and Drugbank databases, respectively, by using the keyword “COVID-19 olfactory impairment”. A total of 5609 potential COVID-19 olfactory impairment-associated targets were obtained after removing duplicate targets and standardizing gene names (Fig. 1).

3.3. Venn diagram of disease gene targets

The R console was used to make Venn diagrams as shown in Figure 2, as well as to obtain 17 drug disease cross targets for LHQW-XYS active ingredients.

3.4. Regulatory network analysis of LHQW-XYS and COVID-19 olfactory impairment-associated targets

The “LHQW-XYS compound–COVID-19 olfactory impairment-target” regulatory network was mapped by

Table 1. Information about the major active compounds in LHQW-XYS.

MOL ID	Name	Degree	Betweenness centrality (BC)	Closeness centrality (CC)
MOL000098	Quercetin	88	0.262263398610901	0.480314960629921
MOL000006	Luteolin	38	0.0655195959488265	0.421658986175115
MOL000422	Kaempferol	35	0.0738647573955381	0.431603773584905
MOL003896	7-Methoxy-2-methylisoflavone	27	0.0108813742961737	0.41590909090909
MOL000173	Wogonin	26	0.0176378338650592	0.414965986394557
MOL002565	Medicarpin	23	0.0134343202863982	0.411235955056179

using Cytoscape 3.9.1 software to match the LHQW-XYS pharmacodynamic components with the 184 action targets obtained from the TCMSP screening. As shown in Figure 3, the network contained 367 nodes and 1764 edges. Additionally, it can be observed from the network that each compound is associated with multiple targets and each target is associated with multiple compounds, and the larger the node, the larger the network value of the target. Additionally, it can be seen that the compound with the highest degree of connectivity is quercetin, which interacts with 770 targets, followed by luteolin (171 targets) and kaempferol (378 targets, Fig. 3).

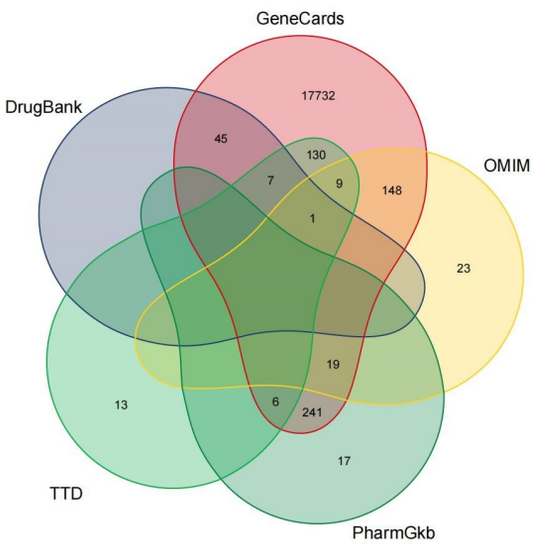


Figure 1. Venn maps of disease targets in different disease databases.

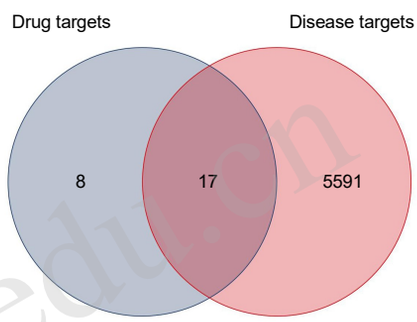


Figure 2. Venn diagram of possible LHQW-XYS targets treated with COVID-19 olfactory impairment.

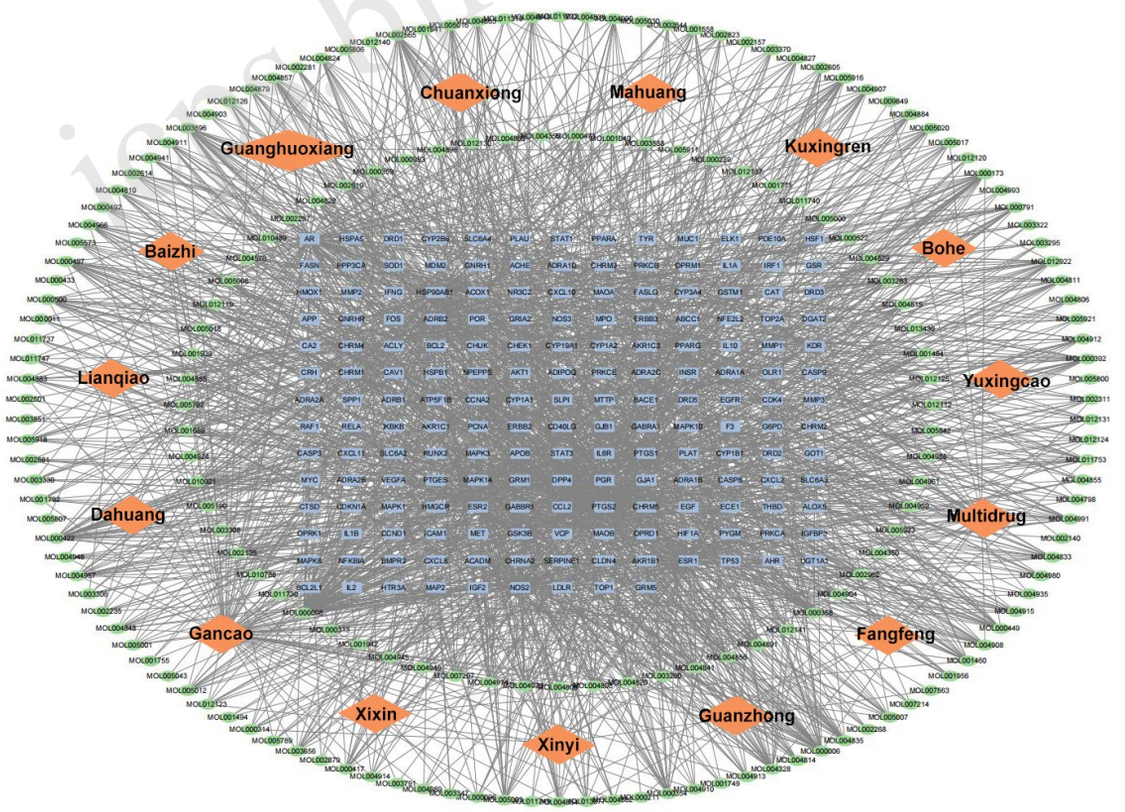


Figure 3. Construction of a target modulation network for COVID-19 olfactory impairment treatment with LHQW-XYS.

3.5. PPI network construction and core module analysis

We imported the intersecting LHQW-XYS and COVID-19 olfactory impairment-associated targets into the STRING 15.0 platform and visualized the PPI network using Cytoscape 3.9.1 software, as shown in Figure 4a. The PPI network consisted of 18 nodes and 31 edges. Targets did not interact with any other targets and were therefore not included in the PPI network. The CytoNCA plugin was used to caulate the degree of connectivity for each target (Fig. 4b). The hub five target genes based on node degree were PPARG, PTGS2, SLC6A4, MAPK14, ACHE, and these target genes may play a key role in the LHQW-XYS mediated therapeutic network.

3.6. GO and KEGG enrichment analyses

As for GO analysis, 250 GO terms were defined, including 212 of BP, 14 of CC, and 23 of MF enriched for these hub target genes. Top five terms of BP, MF, and CC with adjusted *P*-value were presented, respectively (Fig. 5a). The major BP were primarily enriched in regulation of blood pressure, positive regulation of protein transport, response to hypoxia, response to decreased oxygen levels, positive regulation of establishment of protein localization. The major CC showed that the intersection targets were primarily enriched in membrane raft, membrane microdomain. Major MF included peptide binding and amide binding. In addition, 6 KEGG pathways were recognized significantly adjusted *P*-value were presented (Fig. 5b). Results of the KEGG enrichment analysis indicated that the main pathways of the hub genes against COVID-19 olfactory impairment mainly focused on the Serotonergic synapse and Regulation of lipolysis in adipocytes. Therefore, LHQW-XYS might treat COVID-19 olfactory impairment *via* GO terms and pathways above.

3.7. Molecular docking validation

The six LHQW-XYS active components chosen from the PPI network were quercetin, luteolin, kaempferol, 7-methoxy-2-methylisoflavone, wogonin, and medicarpin, with the core targets PTGS2 and, PPARG in the PPI network. The binding energy (Vina score) was used to evaluate the bonding strength between the docked molecules, and the value of the Vina score indicated some binding activity between the proteins and the compounds. The smaller the binding energy (Vina score), the higher the affinity of the receptor and ligand and the more stable the binding of the compound to the target site. The binding energies of the six active ingredients and the corresponding target proteins were all less than –5.0, indicating good docking and high binding activity. Molecular docking simulations showed a stable point docking structure for the binding of small-molecule ligands and protein receptors (Fig. 6).

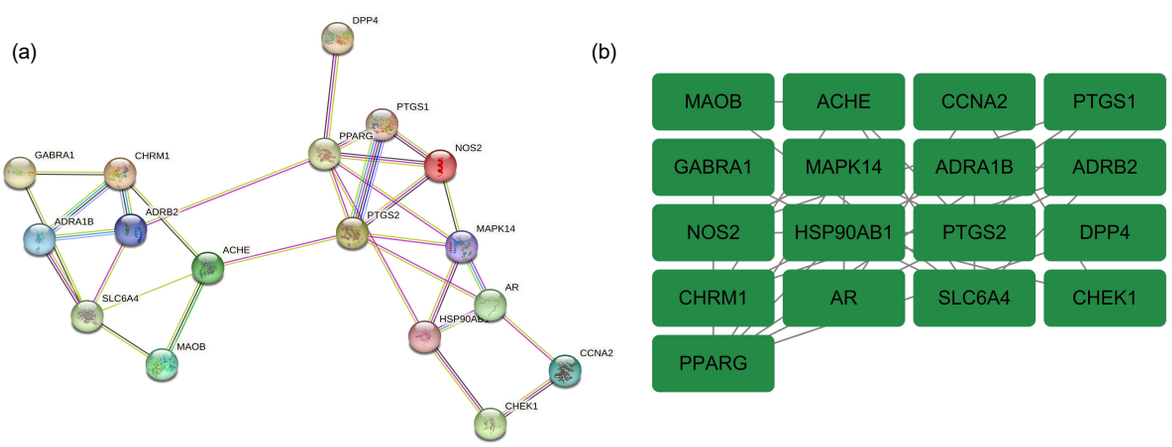
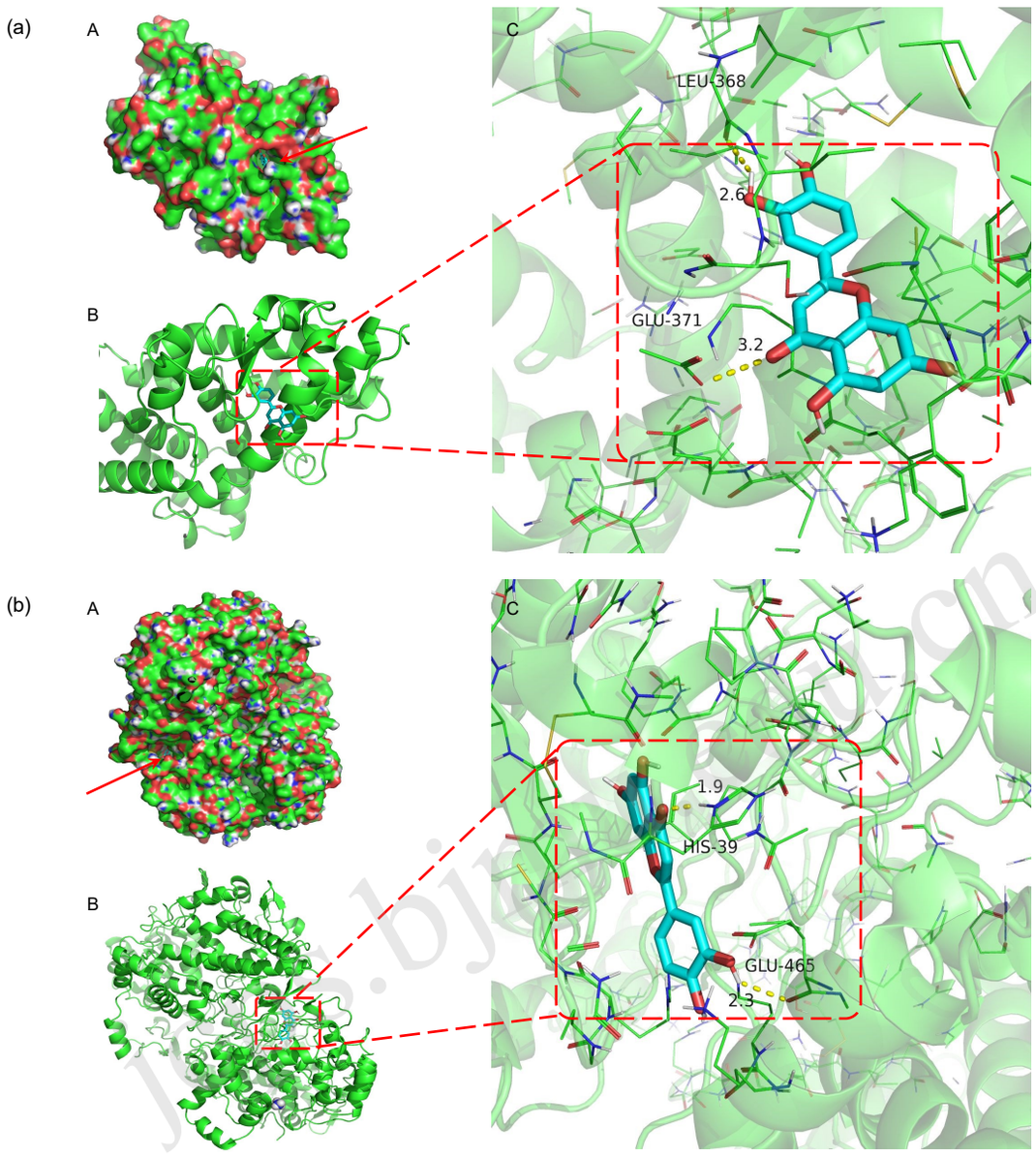


Figure 4. (a) PPI network construction and core module of COVID-19 olfactory impairment and LHQW-XYS; (b) the most significant modules analyzed by the MCODE plugin.

Figure 5. (a) Intersection target GO enrichment analysis: biological process (BP), cell composition (CC), molecular function (MF); (b) KEGG enrichment analysis.



(c)

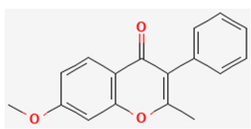
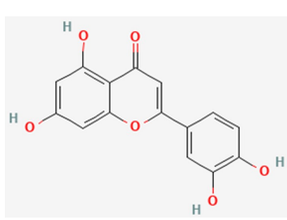
Compound	Molecular formula	Chemical 2D structure	Target gene symbol	Target receptor	Docking results Affinity score (kcal/mol)
7-Methoxy-2-methylisoflavone	$C_{17}H_{14}O_3$		PTGS2; SLC6A4; MAPK14; HSP90AB1; CCNA2	5IKR	–8.3
Luteolin	$C_{15}H_{10}O_6$		PPARG; HSP90AB1; PTGS2	6FZG	–9.5

Figure 6. (a) Schematic diagram of PPARG-6FZG docking; (b) Schematic diagram of PTGS2-5IKR docking; (c) Core active ingredients and core protein binding energy (kcal/mol).

4. Discussion

COVID-19 is a severe acute respiratory syndrome caused by SARS-CoV-2 and is characterized by fever, cough, interstitial pneumonia, malaise, headache, and olfactory disturbance[28]. During the epidemics of SARS-COV and MERS-COV, which are also coronaviruses, olfactory disturbances in patients were rarely reported, but the incidence of olfactory disturbances after SARS-CoV-2 infection is high and severely affects the quality of life of patients[13]. The inability to perceive odors normally with olfactory impairment severely affects nutritional intake, avoidance of harmful fumes and spoiled food, and may lead to malnutrition, weight loss, food poisoning, social isolation, depression, cognitive decline, and even increased mortality[29–31]. Western medicine currently intervenes in the diminished sense of smell (impairment) that occurs after neocoronary pneumonia, mainly based on experience using olfactory exercises, intranasal or oral corticosteroids and intranasal sodium citrate[32]. There are no reliable guidelines or consensus on the efficacy of Western medicine for the treatment of COVID-19 olfactory impairment-associated. The use of corticosteroids is prone to treatment-related complications, such as immunosuppression[33]. Therefore, the French Society of Otolaryngology does not endorse the use of corticosteroids for the treatment of this disease[34]. Therefore, at present, modern medicine is faced with a relatively homogeneous treatment strategy and uncertain efficacy in this regard.

TCM has been instrumental in the fight against the epidemic in China, and one of the representative medicines, Lianhua Qingwen capsule, was recommended as a TCM treatment in the treatment protocol for novel coronavirus pneumonia (6th edition) issued by the National Health Council of China[35]. It has played an important role in the treatment of novel coronavirus pneumonia[36]. It has broad-spectrum antiviral, antibacterial, antipyretic and anti-inflammatory, cough and sputum relieving, and immunomodulatory functions[37]. Studies have shown that LHQW has good therapeutic effects on respiratory diseases caused by various influenza viruses[36]. The pathogenesis of hyposmia after neocoronavirus pneumonia is of increasing concern, and the cause of hyposmia after neocoronavirus pneumonia is closely related to viral damage to olfactory nerves and olfactory cells[38]. Although the respiratory virus is cleared, the virus remains in the cranial tissue, is slow to clear, still has the ability to replicate[39], and can continue to damage the olfactory nerve[40]. LHQW not only inhibits SARS-CoV-2 reproduction[41] and accelerates the removal of the virus to achieve the purpose of treating the root of the disease, but it also contains aromatic and refreshing drugs such as patchouli and peppermint in its composition to relieve nasal congestion, and the whole formula has the effect of treating the symptoms and the root of the disease together.

XYS has a long history of use and is one of the most classic prescriptions for the treatment of nasal disorders. It is commonly used clinically to treat various nasal diseases such as allergic rhinitis, acute and chronic rhinitis, sinusitis, and the accompanying symptoms of hyposmia. In "*Yan's Jisheng Fang*", it is recorded that XYS is used to treat: "Lung deficiency, wind, cold, dampness and heat add up to nasal congestion, incessant snot, or breathlessness, or inability to smell odor". The virus invades the lung, the function of the lung is destroyed, the airflow of the meridians is not smooth, and the nose is blocked, leading to the loss of smell. In XYS, Xin Yi, Sheng Ma, Bai Zhi, Fang Feng, Qiang Huo, Gao Ben, Xi Xin and Chuan Xiong move upward to clear the nasal cavity and benefit the brain, with Mu Tong moving downward to clear the fire in the body and open the middle jiao of the body, and licorice to regulate the middle jiao, working together to play an effective role. The Xin Yi, Bai Zhi, and Xi Xin in XYS all have the effect of relieving nasal congestion,

and modern pharmacological studies have shown that the main medicinal ingredient in Xin Yi, Xin Yi volatile oil, has anti-inflammatory, antibacterial, and central nervous system regulating effects[42]. Fructoside lactone, hydrated oxidized prebiotics and dahurin isolated from Angelica dahurica can significantly inhibit the inflammatory response when administered intraperitoneally to rats[43]. Aqueous decoction of Angelica dahurica has significant anti-inflammatory effects[44]. The volatile oil of Silybum marianum improves the local infiltration of nasal mucosal inflammation[45]. In the recently published "*Expert Consensus on the Treatment of Common Disorders after Nucleic Acid/Antigen Conversion of Novel Coronavirus Infection in Chinese Medicine*", it is stated that the main formula for the evidence of residual evil closing the orifice of reduced olfaction is also selected from XYS[46]. Therefore, the clinical use of LHQW can be used to remove the infectious virus to treat the root of the disease and with XYS to improve nasal congestion to relieve the symptoms.

To further validate the clinical value of LHQW-XYS and to carry out subsequent basic research, a preliminary network pharmacological analysis was done in this study, and 184 active ingredients in LHQW-XYS were found to interact with COVID-19 olfactory impairment-associated target genes by screening active ingredients and targets through the TCMSP database. The three active ingredients with the highest degree of association were quercetin, kaempferol and lignan. Previous studies have shown that quercetin exhibits anti-SARS-CoV-2 activity by inhibiting the interaction of SARS-CoVS protein with ACE2, inhibiting viral protease and uncoupling enzyme activities, as well as inhibiting ACE activity on the host cell side and increasing intracellular zinc levels[47]. Also quercetin has anti-inflammatory properties, and in LPS-induced acute sinusitis in mice, the secretion of IL-6 in the nasal mucosa was significantly reduced after quercetin action, and histopathological and immunofluorescence analyses likewise showed a lower inflammatory response in the nasal mucosa and a lower degree and intensity of IL-1α, COX-2, and IL-10 expression in the epithelium and lamina propria after quercetin use[48].

Lignocaine interferes with SARS-CoV-2 spinosin, major protease and nucleocapsid protein to inhibit viral infection[49,50]. Lignans antagonize the activation of toll-like receptor 4 (TLR4)/TNF receptor-associated factor 6 (TRAF6)/nuclear transcription factor-κB (NF-κB) signaling pathway and reduce inflammation[51]. Lignans exert anti-inflammatory activity through the regulation of transcription factors such as STAT3, NF-κB, and AP-125[52] and can block the polarization of dysplastic microglia and inhibit neuronal cell degeneration[53], and these actions may mediate anti-inflammatory and antioxidant effects to protect neural tissue and other organs from inflammation[51,54]. Recent studies have shown that the selection of palmitoylethanolamide and lignocaine (PEA-LUT) as anti-inflammatory/neuroprotective agents in combination with olfactory training to target patients with olfactory dysfunction after COVID-19 may better promote the recovery of olfactory dysfunction after COVID-19[55].

Kaempferol has potential anti-COVID-19 targets, and bioinformatic screening of kaempferol anti-COVID-19 genes revealed that they were mainly enriched in inflammation (TNF, JUN, etc.) and viral infection (AKT1, JNK, etc.), and that kaempferol treatment significantly reduced the transcript levels of AKT1, JNK and JUN in LPS-activated macrophages[56]. Protein 3a (U274) is the most important helper virus channel-forming protein and plays a crucial role in the release phase of coronavirus particles during infection. Studies have shown that kaempferol has a greater inhibitory effect on the 3a protein of SARS-CoV-2 in

African clawed toad oocytes[57]. When an inflammatory response occurs in the nose, TNF-α expression is significantly increased in the submucosa of the nose[58]. In addition, kaempferol significantly reduced the expression of inflammatory markers (IL-32, TSLP, IL-4, IL-8, ICAM-1, MIP-2 and COX-2)[59].

According to GO and KEGG enrichment results showed that the main pathways involved in LHQW-XYS treatment of COVID-19 olfactory impairment-associated are Serotonergic synapse and Regulation of lipolysis in adipocytes. In the PPI network, Five genes, PPARG, PTGS2, SLC6A4, MAPK14, and ACHE, were found to play key roles in LHQW-XYS treatment of COVID-19 olfactory impairment-associated. The six active ingredients of LHQW-XYS were selected for molecular docking with five central target genes, and the results showed that the core target genes were PTGS2 and PPARG. These active ingredients could freely bind to the core target genes, indicating the efficacy of LHQW-XYS in treating COVID-19 olfactory impairment-associated.

PTGS2 (COX-2), has anti-inflammatory and antioxidant properties through the activation of oxidative metabolites of cox-2-derived ω-3 PUFAs in macrophages[60]. Activation of the immune response occurs after viral infection, which includes the release of inflammatory mediators such as pro-inflammatory cytokines (IL-6, IL-1β, TNF-α) and eicosanoids (prostaglandins and leukotrienes)[61]. COX-2 is responsible for a major part of pg production leading to pain and inflammation, and the SARS coronavirus outbreak in 2003 increased pg production by binding to COX-2[62]. Mast cells release inflammatory mediators that act on the nasal mucosa and cause nasal symptoms during the pathogenesis of rhinitis; therefore, limiting COX-2 expression is particularly important to reduce airway inflammation and improve nasal function in allergic rhinitis[63]. PPARG rapidly senses cellular stress and acts in multiple anti-inflammatory and neuroprotective ways in glial cells, neurons, and cerebrovascular endothelial cells of the central nervous system[64].

It was shown that PPAR-γ is expressed in eosinophils and epithelial cells of the nasal mucosa[65], PPAR-γ messenger RNA and protein expression levels were significantly increased in the nasal mucosa of patients with perennial allergic rhinitis[66] PPARγ agonists highlight their possible role as modulators of inflammatory and immunomodulatory drugs by targeting the cytokine storm in COVID-19 patients[67,68]. By modulating PPARγ expression, the secretion of several pro-inflammatory cytokines, including TNF-α, IL-1 and IL-6, can be reduced in monocytes and macrophages[69]. This effect may counteract the cytokine storm induced by COVID-19 infection[70].

The binding energy of both ligand and receptor is ≤ –5 kcal/mol, and the conformation is stable. The most stable docking with PPARG is the binding of luteolin (–9.5 kcal/mol), and the most stable docking with PTGS2 is the binding of 7-methoxy-2-methylisoflavone (–8.3 kcal/mol). The molecular docking results indicate that the active ingredient binds stably to the receptor protein and can have a therapeutic effect.

5. Conclusions

We have reported for the first time that LHQW-XYS is an effective prescription for the treatment of COVID-19 olfactory infection associated. In addition, promising pharmacological targets have been identified through network pharmacology research, laying the foundation for future clinical research.

Acknowledgements

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References

[1] McFee, R.B. Severe acute respiratory syndrome coronavirus (SARS, SARS CoV). *Disease-a-Month.* **2020**, *66*, 101062.

[2] Abalo-Lojo, J.M.; Pouso-Diz, J.M.; Gonzalez, F. Taste and smell dysfunction in COVID-19 patients. *Ann. Otol. Rhinol. Laryngol.* **2020**, *129*, 1041–1042.

[3] Aggarwal, S.; Garcia-Telles, N.; Aggarwal, G.; Lavie, C.; Lippi, G.; Henry, B.M. Clinical features, laboratory characteristics, and outcomes of patients hospitalized with coronavirus disease 2019 (COVID-19): early report from the United States. *Diagnosis.* **2020**, *7*, 91–96.

[4] Gautier, J.F.; Ravussin, Y. A new symptom of COVID-19: loss of taste and smell. *Obesity.* **2020**, *28*, 848.

[5] Gilani, S.; Roditi, R.; Naraghi, M. COVID-19 and anosmia in Tehran, Iran. *Med. Hypotheses.* **2020**, *141*, 109757.

[6] Hopkins, C.; Surda, P.; Whitehead, E.; Kumar, B.N. Early recovery following new onset anosmia during the COVID-19 pandemic – an observational cohort study. *J. Otolaryngol. Head Neck Surg.* **2020**, *49*, 26.

[7] Huang, C.L.; Wang, Y.M.; Li, X.W.; Ren, L.L.; Zhao, J.P.; Hu, Y.; Zhang, L.; Fan, G.H.; Xu, J.Y.; Gu, X.Y.; Cheng, Z.S.; Yu, T.; Xia, J.A.; Wei, Y.; Wu, W.J.; Xie, X.L.; Yin, W.; Li, H.; Liu, M.; Xiao, Y.; Gao, H.; Guo, L.; Xie, J.G.; Wang, G.F.; Jiang, R.M.; Gao, Z.C.; Jin, Q.; Wang, J.W.; Cao, B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* **2020**, *395*, 497–506.

[8] Merza, M.A.; Haleem Al Mezori, A.A.; Mohammed, H.M.; Abdulah, D.M. COVID-19 outbreak in Iraqi Kurdistan: the first report characterizing epidemiological, clinical, laboratory, and radiological findings of the disease. *Diabetes Metab. Syndr.* **2020**, *14*, 547–554.

[9] Lechien, J.R.; Chiesa-Estomba, C.M.; De Siati, D.R.; Horoi, M.; Le Bon, S.D.; Rodriguez, A.; Dequanter, D.; Blecic, S.; El Afia, F.; Distinguin, L.; Chekkoury-Idrissi, Y.; Hans, S.; Delgado, I.L.; Calvo-Henriquez, C.; Lavigne, P.; Falanga, C.; Barillari, M.R.; Cammaroto, G.; Khalife, M.; Leich, P.; Souchay, C.; Rossi, C.; Journe, F.; Hsieh, J.; Edjlali, M.; Carlier, R.; Ris, L.; Lovato, A.; De Filippis, C.; Coppee, F.; Fakhry, N.; Ayad, T.; Saussez, S. Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. *Eur. Arch. Otorhinolaryngol.* **2020**, *277*, 2251–2261.

[10] Beltrán-Corbellini, Á.; Chico-García, J.L.; Martínez-Poles, J.; Rodríguez-Jorge, F.; Natera-Villalba, E.; Gómez-Corral, J.; Gómez-López, A.; Monreal, E.; Parra-Díaz, P.; Cortés-Cuevas, J.L.; Galán, J.C.; Fragola-Arnau, C.; Porta-Etessam, J.; Masjuan, J.; Alonso-Cánovas, A. Acute-onset smell and taste disorders in the context of COVID-19: a pilot multicentre polymerase chain reaction based case-control study. *Eur. J. Neurol.* **2020**, *27*, 1738–1741.

[11] Brann, D.H.; Tsukahara, T.; Weinreb, C.; Lipovsek, M.; Van den Berge, K.; Gong, B.Y.; Chance, R.; MacAulay, I.C.; Chou, H.J.; Fletcher, R.B.; Das, D.; Street, K.; de Bezieux, H.R.; Choi, Y.G.; Risso, D.; Dudoit, S.; Purdom, E.; Mill, J.; Hachem, R.A.; Matsunami, H.; Logan, D.W.; Goldstein, B.J.; Grubb, M.S.; Ngai, J.; Datta, S.R. Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia. *Sci. Adv.* **2020**, *6*, eabc5801.

[12] Eliezer, M.; Hautefort, C.; Hamel, A.L.; Verillaud, B.; Herman, P.; Houdart, E.; Eloit, C. Sudden and complete olfactory loss of function as a possible symptom of COVID-19. *JAMA Otolaryngol. Head Neck Surg.* **2020**, *146*, 674–675.

[13] Gorzkowski, V.; Bevilacqua, S.; Charmillon, A.; Jankowski, R.; Gallet, P.; Rumeau, C.; Nguyen, D. Evolution of olfactory disorders in COVID-19 patients. *Laryngoscope.* **2020**, *130*, 2667–2673.

[14] Jung, A.Y.; Kim, Y.H. Reversal of olfactory disturbance in allergic rhinitis related to OMP suppression by intranasal budesonide treatment. *Allergy Asthma Immunol. Res.* **2020**, *12*, 110.

[15] Wong, S.K.; Li, W.H.; Moore, M.J.; Choe, H.; Farzan, M. A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. *J. Biol. Chem*. **2004**, *279*, 3197–3201.

[16] Miwa, T.; Ikeda, K.; Ishibashi, T.; Kobayashi, M.; Kondo, K.; Matsuwaki, Y.; Ogawa, T.; Shiga, H.; Suzuki, M.; Tsuzuki, K.; Furuta, A.; Motoo, Y.; Fujieda, S.; Kurono, Y. Clinical practice guidelines for the management of olfactory dysfunction - Secondary publication. *Auris Nasus Larynx.* **2019**, *46*, 653–662.

[17] Shen, X.H.; Yin, F.G. The mechanisms and clinical application of Traditional Chinese Medicine Lianhua-Qingwen capsule. *Biomed. Pharmacother*. **2021**, *142*, 111998.

[18] Ru, J.L.; Li, P.; Wang, J.N.; Zhou, W.; Li, B.H.; Huang, C.; Li, P.D.; Guo, Z.H.; Tao, W.Y.; Yang, Y.F.; Xu, X.; Li, Y.; Wang, Y.H.; Yang, L. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *J. Cheminform*. **2014**, *6*, 13.

[19] Mu, C.L.; Sheng, Y.F.; Wang, Q.; Amin, A.; Li, X.G.; Xie, Y.Q. Potential compound from herbal food of Rhizoma Polygonati for treatment of COVID-19 analyzed by network pharmacology: viral and cancer signaling mechanisms. *J. Funct. Foods.* **2021**, *77*, 104149.

[20] Pei, T.L.; Zheng, C.L.; Huang, C.; Chen, X.T.; Guo, Z.H.; Fu, Y.X.; Liu, J.L.; Wang, Y.H. Systematic understanding the mechanisms of vitiligo pathogenesis and its treatment by Qubaibabuqi formula. *J. Ethnopharmacol*. **2016**, *190*, 272–287.

[21] Schoch, C.; Ciufo, S.; Domrachev, M.; Hotton, C.; Kannan, S.; Khovanskaya, R.; Leipe, D.; McVeigh, R.; O'Neill, K.; Robbertse, B.; Sharma, S.; Soussov, V.; Sullivan, J.P.; Sun, L.; Turner, S.; Karsch-Mizrachi, I. NCBI Taxonomy: a comprehensive update on curation, resources and tools. *Database (Oxford)*. **2020**, *baaa062*, 1–21.

[22] Doncheva, N.T.; Morris, J.H.; Gorodkin, J.; Jensen, L.J. Cytoscape StringApp: network analysis and visualization of proteomics data. *J. Proteome Res*. **2019**, *18*, 623–632.

[23] Chin, C.H.; Chen, S.H.; Wu, H.H.; Ho, C.W.; Ko, M.T.; Lin, C.Y. cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst. Biol*. **2014**, *8*, S11.

[24] Zhou, J.G.; Xiong, W.; Wang, Y.; Guan, J.H. Protein function prediction based on PPI networks: network reconstruction vs edge enrichment. *Front. Genet*. **2021**, *12*, 758131.

[25] Kontoyianni, M. Docking and virtual screening in drug discovery. *Methods Mol. Biol*. **2017**, *1647*, 255–266.

[26] El-Hachem, N.; Haibe-Kains, B.; Khalil, A.; Kobeissy, F.H.; Nemer, G. AutoDock and AutoDockTools for protein-ligand docking: beta-site amyloid precursor protein cleaving enzyme 1(BACE1) as a case study. *Methods Mol. Biol*. **2017**, *1598*, 391–403.

[27] Rosignoli, S.; Paiardini, A. Boosting the full potential of PyMOL with structural biology plugins. *Biomolecules.* **2022**, *12*, 1764.

[28] Guan, W.J.; Ni, Z.Y.; Hu, Y.; Liang, W.H.; Ou, C.Q.; He, J.X.; Liu, L.; Shan, H.; Lei, C.L.; Hui, D.S.C.; Du, B.; Li, L.J.; Zeng, G.; Yuen, K.Y.; Chen, R.C.; Tang, C.L.; Wang, T.; Chen, P.Y.; Xiang, J.; Li, S.Y.; Wang, J.L.; Liang, Z.J.; Peng, Y.X.; Wei, L.; Liu, Y.; Hu, Y.H.; Peng, P.; Wang, J.M.; Liu, J.Y.; Chen, Z.; Li, G.; Zheng, Z.J.; Qiu, S.Q.; Luo, J.; Ye, C.J.; Zhu, S.Y.; Zhong, N.S. China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl. J. Med*. **2020**, *382*, 1708–1720.

[29] Gopinath, B.; Anstey, K.J.; Kifley, A.; Mitchell, P. Olfactory impairment is associated with functional disability and reduced independence among older adults. *Maturitas.* **2012**, *72*, 50–55.

[30] Pinto, J.M.; Wroblewski, K.E.; Kern, D.W.; Schumm, L.P.; McClintock, M.K. Olfactory dysfunction predicts 5-year mortality in older adults. *PLoS One.* **2014**, *9*, e107541.

[31] Glezer, I.; Bruni-Cardoso, A.; Schechtman, D.; Malnic, B. Viral infection and smell loss: The case of COVID-19. *J. Neurochem*. **2021**, *157*, 930–943.

[32] Najafloo, R.; Majidi, J.; Asghari, A.; Aleemardani, M.; Kamrava, S.K.; Simorgh, S.; Seifalian, A.; Bagher, Z.; Seifalian, A.M. Mechanism of anosmia caused by symptoms of COVID-19 and emerging treatments. *ACS Chem. Neurosci*. **2021**, *12*, 3795–3805.

[33] Ahmed, A.K.; Sayad, R.; Mahmoud, I.A.; Abd EL-Monem, A.M.; Badry, S.H.; Ibrahim, I.H.; Hafez, M.H.; El-Mokhtar, M.A.; Sayed, I.M. "Anosmia" the mysterious collateral damage of COVID-19. *J. NeuroVirology.* **2022**, *28*, 189–200.

[34] Russell, B.; Moss, C.; Rigg, A.; Van Hemelrijck, M. COVID-19 and treatment with NSAIDs and corticosteroids: should we be limiting their use in the clinical setting? *Ecancermedicalscience.* **2020**, *14*, 1023.

[35] Zeng, Z.; Lan, T.X.; Wei, Y.Q.; Wei, X.W. CCL5/CCR5 axis in human diseases and related treatments. *Genes Dis*. **2022**, *9*, 12–27.

[36] Zhang, X.L.; Cao, D.; Liu, J.N.; Zhang, Q.; Liu, M.J. Efficacy and safety of Lianhua Qingwen combined with conventional antiviral Western Medicine in the treatment of coronavirus disease (covid-19) in 2019: protocol for a systematic review and meta-analysis. *Medicine.* **2020**, *99*, e21404.

[37] CDC Weekly, C. Protocol for prevention and control of COVID-19 (edition 6). *China CDC Week*. **2020**, *2*, 321–326.

[38] Ziuzia-Januszewska, L.; Januszewski, M. Pathogenesis of olfactory disorders in COVID-19. *Brain Sci*. **2022**, *12*, 449.

[39] Stein, S.R.; Ramelli, S.C.; Grazioli, A.; Chung, J.Y.; Singh, M.; Yinda, C.K.; Winkler, C.W.; Sun, J.; Dickey, J.M.; Ylaya, K.; Ko, S.H.; Platt, A.P.; Burbelo, P.D.; Quezado, M.; Pittaluga, S.; Purcell, M.; Munster, V.J.; Belinky, F.; Ramos-Benitez, M.J.; Boritz, E.A.; Lach, I.A.; Herr, D.L.; Rabin, J.; Saharia, K.K.; Madathil, R.J.; Tabatabai, A.; Soherwardi, S.; McCurdy, M.T. NIH COVID-19 Autopsy Consortium.; Peterson, K.E.; Cohen, J.I.; de Wit, E.; Vannella, K.M.; Hewitt, S.M.; Kleiner, D.E.; Chertow, D.S. SARS-CoV-2 infection and persistence in the human body and brain at autopsy. *Nature*. **2022**, *612*, 758–763.

[40] Kandemirli, S.G.; Altundag, A.; Yildirim, D.; Tekcan Sanli, D.E.; Saatci, O. Olfactory bulb MRI and paranasal sinus CT findings in persistent COVID-19 anosmia. *Acad. Radiol*. **2021**, *28*, 28–35.

[41] Liu, M.; Gao, Y.; Yuan, Y.; Yang, K.L.; Shi, S.Z.; Tian, J.H.; Zhang, J.H. Efficacy and safety of herbal medicine (Lianhuaqingwen) for treating COVID-19: a systematic review and meta-analysis. *Integr. Med. Res*. **2021**, *10*, 100644.

[42] Wang, P.; Zhang, H.Y.; Liu, Y.M. Research progress on chemical constituents, Pharmacological effects and clinical application of volatile oil From Magnolia liliflora. *China Pharmacy*. **2022**, *33*, 378–384.

[43] Kimura, Y.; Okuda, H.; Baba, K. Histamine-release effectors from *Angelicadahurica* var. *Dahurica* Root. *J. Nat. Prod*. **1997**, *60*, 249–251.

[44] Zou, J.Y.; Su, W.; Pan, Y.; Cui, J. Chemical Components and Pharmacological Action for Angelica dahurica Sinensis and Predictive Analysis on its Q-marker. *Modernization of Traditional Chinese Medicine and Materia Medica-World Science and Technology*. This article can be found online at http://kns.cnki.net/kcms/detail/11.5699.R.20230914.1254.004.html.

[45] Hu, Y.L. Preliminary study on effects of Xixin oil on blood histamine and pathomorphological change in nasal mucosa in Guinea pigs with allergic rhinitis. *Chin. J. Exp. Tradit. Med. Form*. **2011**, *17*, 149–151.

[46] Zhang, X. The China Association of Chinese Medicine released the expert consensus on the treatment of common diseases after COVID-19 became negative with traditional Chinese medicine. *J. Tradit. Chin. Med. Manage*. **2023**, *31*, 7.

[47] Prasansuklab, A.; Theerasri, A.; Rangsinth, P.; Sillapachaiyaporn, C.; Chuchawankul, S.; Tencomnao, T. Anti-COVID-19 drug candidates: a review on potential biological activities of natural products in the management of new coronavirus infection. *J. Tradit. Complement. Med*. **2021**, *11*, 144–157.

[48] Tiboc-Schnell, C.N.; Filip, G.A.; Man, S.C.; Decea, N.; Moldovan, R.; Opris, R.; Sas, V.; Tabaran, F. Quercetin attenuates naso-sinusal inflammation and inflammatory response in lungs and brain on an experimental model of acute rhinosinusitis in rats. *J. Physiol. Pharmacol*. **2020**, *71*, 10.26402/jpp.2020.4.03.

[49] Theoharides, T.C. COVID-19, pulmonary mast cells, cytokine storms, and beneficial actions of luteolin. *BioFactors*. **2020**, *46*, 306–308.

[50] Shawan, M.M.A.K.; Halder, S.K.; Hasan, M.A. Luteolin and abyssinone II as potential inhibitors of SARS-CoV-2: an in silico molecular modeling approach in battling the COVID-19 outbreak. *Bull Natl. Res. Cent*. **2021**, *45*, 27.

[51] Di Stadio, A.; D'Ascanio, L.; Vaira, L.A.; Cantone, E.; De Luca, P.; Cingolani, C.; Motta, G.; De Riu, G.; Vitelli, F.; Spriano, G.; De Vincentiis, M.; Camaioni, A.; La Mantia, I.; Ferreli, F.; Brenner, M.J. Ultramicronized palmitoylethanolamide and luteolin supplement combined with olfactory training to treat post-COVID-19 olfactory impairment: a multi-center double-blinded randomized placebo- controlled clinical trial. *Curr. Neuropharmacol*. **2022**, *20*, 2001–2012.

[52] Yang, Y.; Tan, X.; Xu, J.G.; Wang, T.Y.; Liang, T.Y.; Xu, X.; Ma, C.C.; Xu, Z.M.; Wang, W.J.; Li, H.Y.; Shen, H.T.; Li, X.; Dong, W.L.; Chen, G. Luteolin alleviates neuroinflammation via downregulating the TLR4/TRAF6/NF-κB pathway after intracerebral hemorrhage. *Biomed Pharmacother*. **2020**, *126*, 1–12.

[53] Aziz, N.; Kim, M.Y.; Cho, J.Y. Anti-inflammatory effects of luteolin: a review of *in vitro*, *in vivo*, and in silico studies. *J. Ethnopharmacol*. **2018**, *225*, 342–358.

[54] Nabavi, S.F.; Braidy, N.; Gortzi, O.; Sobarzo-Sanchez, E.; Daglia, M.; Skalicka-Woźniak, K.; Nabavi, S.M. Luteolin as an anti-inflammatory and neuroprotective agent: a brief review. *Brain Res. Bull*. **2015**, *119*, 1–11.

[55] Lee, M.H.; Perl, D.P.; Nair, G.; Li, W.X.; Maric, D.; Murray, H.; Dodd, S.J.; Koretsky, A.P.; Watts, J.A.; Cheung, V.; Masliah, E.; Horkayne-Szakaly, I.; Jones, R.; Stram, M.N.; Moncur, J.; Hefti, M.; Folkerth, R.D.; Nath, A. Microvascular injury in the brains of patients with covid-19. *N Engl. J. Med*. **2021**, *384*, 481–483.

[56] Sun, Y.X.; Tao, Q.; Cao, Y.; Yang, T.T.; Zhang, L.; Luo, Y.F.; Wang, L. Kaempferol has potential anti-coronavirus disease 2019 (COVID-19) targets based on bioinformatics analyses and pharmacological effects on endotoxin-induced cytokine storm. *Phytother. Res*. **2023**, *37*, 2290–2304.

[57] Ahmadian, R.; Rahimi, R.; Bahramsoltani, R. Kaempferol: an encouraging flavonoid for COVID-19. *Boletin Latinoamericano Y Del Caribe De Plantas Med. Y Aromat*. **2020**, *19*, 492–494.

[58] Jung, H.W.; Jung, J.K.; Cho, C.W.; Kang, J.S.; Park, Y.K. Antiallergic effect of KOB03, a polyherbal medicine, on mast cell-mediated allergic responses in ovalbumin-induced allergic rhinitis mouse and human mast cells. *J. Ethnopharmacol*. **2012**, *142*, 684–693.

[59] Oh, H.A.; Han, N.R.; Kim, M.J.; Kim, H.M.; Jeong, H.J. Evaluation of the effect of kaempferol in a murine allergic rhinitis model. *Eur. J. Pharmacol*. **2013**, *718*, 48–56.

[60] Groeger, A.L.; Cipollina, C.; Cole, M.P.; Woodcock, S.R.; Bonacci, G.; Rudolph, T.K.; Rudolph, V.; Freeman, B.A.; Schopfer, F.J. Cyclooxygenase-2 generates anti-inflammatory mediators from omega-3 fatty acids. *Nat. Chem. Biol*. **2010**, *6*, 433–441.

[61] Ricke-Hoch, M.; Stelling, E.; Lasswitz, L.; Gunesch, A.P.; Kasten, M.; Zapatero-Belinchón, F.J.; Brogden, G.; Gerold, G.; Pietschmann, T.; Montiel, V.; Balligand, J.L.; Facciotti, F.; Hirsch, E.; Gausepohl, T.; Elbahesh, H.; Rimmelzwaan, G.F.; Höfer, A.; Kühnel, M.P.; Jonigk, D.; Eigendorf, J.; Tegtbur, U.; Mink, L.; Scherr, M.; Illig, T.; Schambach, A.; Pfeffer, T.J.; Hilfiker, A.; Haverich, A.; Hilfiker-Kleiner, D. Impaired immune response mediated by prostaglandin E2 promotes severe COVID-19 disease. *PLoS One.* **2021**, *16*, e0255335.

[62] FitzGerald, G.A. Misguided drug advice for COVID-19. *Science.* **2020**, *367*, 1434.

[63] Qi, L.J.; Wang, R.Z.; Gao, S.; Chen, X.J.; Zhang, X.; Zhang, Y.P. Molecular mechanisms underlying the effects of bimin Kang mixture on allergic rhinitis: network pharmacology and RNA sequencing analysis. *Biomed Res*. *Int*. **2022**, *2022*, 7034078.

[64] Gold, P.W. The PPARg system in major depression: pathophysiologic and therapeutic implications. *Int*. *J*. *Mol*. *Sci*. **2021**, *22*, 9248.

[65] Fukui, N.; Honda, K.; Ito, E.; Ishikawa, K. Peroxisome proliferator-activated receptor γ negatively regulates allergic rhinitis in mice. *Allergol*. *Int*. **2009**, *58*, 247–253.

[66] Kang, H.J.; Cinn, Y.G.; Hwang, S.J.; Won Chae, S.; Woo, J.S.; Lee, S.H.; Lee, H.M. Up-regulation of peroxisome proliferator-activated receptor γ in perennial allergic rhinitis. *Arch*. *Otolaryngol*. **2006**, *132*, 1196–1200.

[67] Carboni, E.; Carta, A.R.; Carboni, E. Can pioglitazone be potentially useful therapeutically in treating patients with COVID-19? *Med*. *Hypotheses*. **2020**, *140*, 109776.

[68] Mukherjee, J.J.; Gangopadhyay, K.K.; Subir, R. Use of pioglitazone in people with type 2 diabetes mellitus with coronavirus disease 2019 (COVID-19): boon or bane? *Diabetes Metab*. *Syndr*. **2020**, *14*, 829–831.

[69] Josep, B.R.; Rong, S.; Roberts Paul, C.; Raquel, H. PPAR-γ activation as an anti-inflammatory therapy for respiratory virus infections. *Viral Immunol*. **2010**, *23*, 343–352.

[70] Francisqueti-Ferron, F.V.; Garcia, J.L.; Ferron, A.J.T.; Nakandakare-Maia, E.T.; Gregolin, C.S.; das Chagas Silva, J.P.; dos Santos, K.C.; Lo, Â.T.C.; Siqueira, J.S.; de Mattei, L.; de Paula, B.H.; Sarzi, F.; de Almeida Silva, C.C.V.; Moreto, F.; Costa, M.R.; Ferreira, A.L.A.; Minatel, I.O.; Corrêa, C.R. Gamma-oryzanol as a potential modulator of oxidative stress and inflammation via PPAR-y in adipose tissue: a hypothetical therapeutic for cytokine storm in COVID-19? *Mol*. *Cell Endocrinol*. **2021**, *520*, 111095.

网络药理学研究揭示连花清瘟-辛夷散联合治疗新冠后嗅觉损伤的潜在作用机制

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摘要: 本文旨在利用网络药理学方法探索连花清瘟-辛夷散对新冠嗅觉损伤主要成分的作用靶点, 并试图揭示其在新冠致嗅觉损伤相关治疗中的作用机制。我们利用TCMSP平台进行口服利用度和类药性筛选获取潜在有效成分; Swiss TargetPrediction平台预测有效成分的作用靶标, 构建药物-成分-作用靶标网络, 再通过GeneCards、OMIM、TTD平台获取新冠嗅觉损伤基因靶点, 将药物靶标和疾病基因相交集获得共同靶点。利用STRING及Cytoscape 3.8.2软件构建靶标-疾病基因PPI网络, 筛选关键靶点和核心基因团簇, 借助Metascape平台对关键靶点进行GO和KEGG富集分析, 并就筛选出来的核心有效成分及其作用靶标映射到通路中, 构建核心有效成分-靶标-通路网络, 最后进行分子对接。结果显示连花清瘟-辛夷散活性成分有4669个潜在靶点, 5609个疾病靶点和17个药物-疾病交叉靶点。GO和KEGG富集分析显示, 连花清瘟-辛夷散治疗新冠嗅觉损伤相关的机制可能是由于相关信号通路的调控作用, 如5-羟色胺能突触和脂肪细胞的脂解调节。分子对接结果表明, 6种活性成分(槲皮素、木犀草素、山奈酚、7-甲氧基-2-甲基异黄酮、汉黄芩素、美迪紫檀素)和2个关键基因(PTGS2、PPARG)具有良好的结合特性。综上我们可以得到结论: 连花清瘟-辛夷散可能通过作用于脂肪细胞中的5-羟色胺能突触和调节脂解通路, 从而实现治疗新冠嗅觉损伤的相关作用。

关键词: 连花清瘟-辛夷散; 新冠相关嗅觉损伤; 网络药理学; 分子对接