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Exploring the anti novel coronavirus mechanism of Atractylodes Rhizoma based on network pharmacology and molecular docking

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Abstract: *Objective: To explore the potential mechanism of Atractylodes Rhizoma against COVID-19 by network pharmacology and molecular docking. Method Retrieval of Active Components and Targets in Atractylodes Rhizoma with TCMSP Database, Screening related targets of COVID-19 through GeneCards and UniProt databases, and querying the genes corresponding to the targets. Intersect drug prediction target and disease target, and draw Protein-Protein Interaction Network(PPI) through STRING database. Use DAVID database to analyze GO function enrichment and KEGG pathway of target. Then, the network diagram of "disease pathway target component drug" was constructed by using the software Cytoscape 3.9.1. Finally, download the protein receptor structure related to COVID-19 from the protein PDB database, and use the active component of Atractylodes Rhizoma as the ligand, and molecular docking with SARS-CoV-2 3CL, SARS-CoV-2 3CLpro and ACE2. Results we obtained 27 targets and 141 KEGG signal pathways of Atractylodes Rhizoma for COVID-19, mainly involving human cytomegalovirus infection, IL-17 signal pathway, TNF signal pathway and so on. The results of molecular docking showed that the main active compounds in Atractylodes Rhizoma, such as wogonin, NSC63551and Stigmasterol 3-O-beta-D-glucopyranoside, had good binding activity to COVID-19 related protein receptors. Conclusion Speculation the active compounds in Atractylodes Rhizoma may regulate multiple signal pathways by binding with ACE2, SARS-CoV-2 3CL and SARS-CoV-2 3CLpro to targets such as NOS2, AR, SCN5A, PPARG, IL-6, DPP4, and thus play the role of anti novel coronavirus.*

Keywords: *Atractylodes Rhizoma, novel coronavirus, network pharmacology, molecular docking*

1. Introduction

In 2019, the novel coronavirus pneumonia (COVID-19) was found to be caused by a severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) [1-3], which is the seventh known coronavirus to infect humans [4] and can cause severe symptoms and even death, although it has been nearly three years since the outbreak, But it continues to wreak havoc across the globe. At present, most of the treatment methods are assisted by traditional Chinese medicine.

The dry rhizoma of Atractylodes lancea(Thunb.)DC. or Atractylodes chinensis(DC.)Koidz., a composite plant, is pungent, bitter, warm, and belongs to the spleen, stomach, and liver meridians[5]. It is a traditional Chinese medicine with homology of food. Atractylodes Rhizoma played an important role in the treatment of plague in ancient times [6]. Now, in the fight against the novel coronavirus epidemic, it is also widely used in the prevention and treatment of pneumonia infected by the novel coronavirus, and it is one of the most used Chinese herbs [7]. Atractylodes Rhizoma is rich in aromatic volatile oil. Since Zhang Zhongjing of the Han Dynasty, there have been custom records of burning smoke for epidemic prevention, which makes use of the epidemic prevention effect of volatile oil in Atractylodes Rhizoma.

Due to the multi-component, multi-pathway and multi-target nature of traditional Chinese medicine, the anti COVID-19 mechanism of Atractylodes Rhizoma remains unclear. Therefore, this study intends to screen the potential active components of Atractylodes lancea in the treatment of COVID-19 through network pharmacology and molecular docking virtual screening. To construct the network relationship model of Atractylodes Rhizoma " disease-pathway-target-component-drug", so as to provide reference for the further study of Atractylodes Rhizoma and the treatment of COVID-19.

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2. Materials and methods

2.1 Database and software

TCMSP(http://tcmspw.com/tcmsp.php);PDB(https://www.rcsb.org/);Uniprot(https://www.uniprot.org);GeneCards(https://www.genecards.org);DAVID(https://david.ncifcrf.gov); STRING(https://string-db.org); Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/); Weishengxin Online drawing website(www.bioinformatics.com.cn); Cytoscape3.9.1; AutoDock Tools 1.5.7; PyMOL; LigPlot+.

2.2 Screening of active ingredients of Atractylodes Rhizoma and acquisition of target

By searching the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) with Analysis Platform, the active compound of Atractylodes Rhizoma and the potential targets were obtained. The target proteins were screened with oral bioavailability (OB)≥30 % and drug-like drug (DL)≥0.18. In the UniProt database, enter the protein name and correct the target to the official gene symbol.

2.3 Prediction of potential targets in Atractylodes Rhizoma treatment of COVID-19

In Genecards database, use "Coronavirus disease 2019(COVID-19)" as the key word to search for COVID-19 related targets [8]. Exclusion of duplicated genes, the targets of Atractylodes Rhizoma and those related to COVID-19 were intersected by Venny2.1.0, and the common genes obtained were potential targets of atractylodes for the treatment of COVID-19.

2.4 Construct protein interaction network

The intersection targets were imported into the STRING database, and the species were defined as "Homo sapiens" to construct the protein interaction network.

2.5 Gene ontology (GO) enrichment analysis and KEGG pathway analysis

DAVID database was used for GO function and KEGG pathway enrichment analysis of targets. Weishengxin online tool was used to visualize the enrichment analysis results. The threshold value was set to the $P<0.05$ and select the top 20 enrichment items ranked by GO and KEGG.

2.6 Disease-Pathway-target-component-drug network

According to the above data of relevant active ingredients and targets, analyze the first 20 key signal pathways of Atractylodes Rhizoma in the treatment of COVID-19. Then, Cytoscape 3.9.1 software was used to construct a "disease-pathway-target-component-drug" network to analyze the mechanism of active components of Atractylodes Rhizoma in the treatment of COVID-19.

2.7 Molecular docking

Download the “mol2” format of the active ingredients of Atractylodes Rhizoma through TCMSP database, import the AutoDock Tools 1.5.7 software, conduct hydrogenation, select them as ligands, detect the twist keys and centers, and save them in pdbqt format. Consult relevant literature and download SARS-CoV-2 3CL pro(PDB ID: 6XHU)[9], ACE2(PDB ID: 1R4l)[10-11], SARS-CoV-2 3CL(PDB ID: PDB ID: 6LU7)[12-13] in “PDB” format. Open in Autodock, delete water molecules, hydrogenation, and calculate point charges, and save them in “pdbqt” format. Finally, the selected active components of Atractylodes Rhizoma were subjected to molecular docking with receptor proteins. After the docking was completed, cluster analysis was carried out on the results of molecular docking with Auto-Dock plates, and compared the free energy of docking combined. Pymol and LigPlot+ software were used for visual analysis and processing of the optimal conformation.

3. Conclusion

3.1 Atractylodes Rhizoma and COVID-19 potential targets

According to the criteria of OB≥30% and DL≥0.18, 9 active ingredients were screened from

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Atractylodes Rhizoma (Table 1, Figure 1). Through UniProt database, 67 potential targets of Atractylodes Rhizoma were obtained by inputting protein names. A total of 324 COVID-19 related targets were collected from the Genecards database.

Table 1: Active ingredients of Atractylodes Rhizoma

MOL ID	Compound name	OB/%	DL
MOL000173	wogonin	30.68	0.23
MOL000179	2-Hydroxyisoxypropyl-3-hydroxy-7-isopentene-2,3-dihydrobenzofuran-5-carboxylic	45.2	0.20
MOL000184	NSC63551	39.25	0.76
MOL000186	Stigmasterol 3-O-beta-D-glucopyranoside_qt	43.83	0.76
MOL000188	3β-acetoxyatractylone	40.57	0.22
MOL000085	beta-daucosterol_qt	36.91	0.75
MOL000088	beta-sitosterol 3-O-glucoside_qt	36.91	0.75
MOL000092	daucosterin_qt	36.91	0.76
MOL000094	daucosterol_qt	36.91	0.76

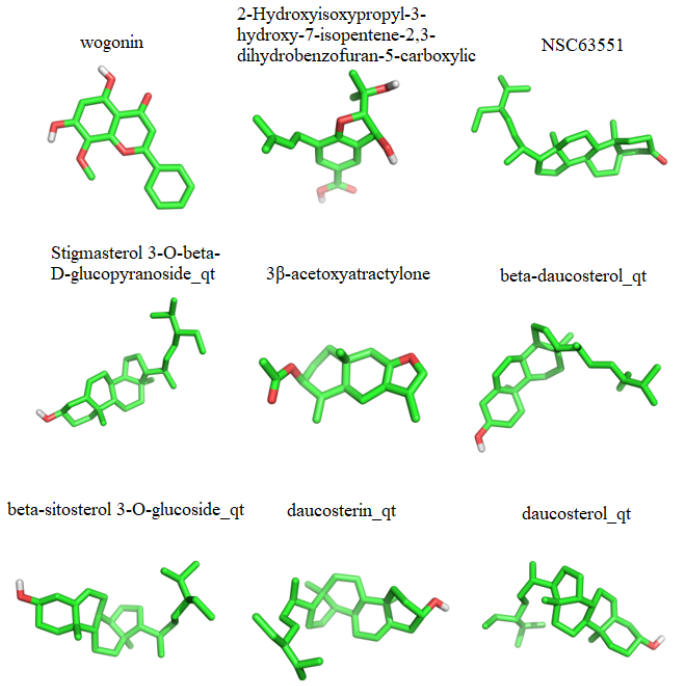

Figure 1: Structure of active ingredients in Atractylodes Rhizoma

3.2 Potential target of Atractylodes Rhizoma in the treatment of COVID-19

Venny2.1.0 online tool was used to screen out 27 common targets of Atractylodes Rhizoma and COVID-19, the results are shown in Figure 2. The 27 intersection targets were imported into the STRING database, the species was defined as "Homo sapiens", and the selection score was > 0.7(high confidence score) to construct the protein interaction (PPI) network, as shown in Figure 3. The main intersection targets between Atractylodes Rhizoma and COVID-19 are NOS2, AR, SCN5A, PPARG, IL-6, DPP4, etc.

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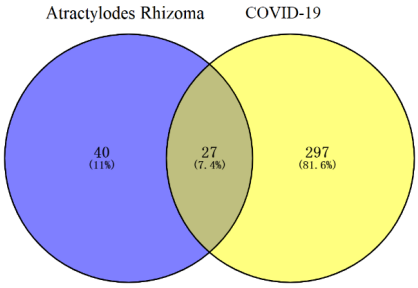

Figure 2: Venn diagram of Atractylodes Rhizoma and COVID-19 targets

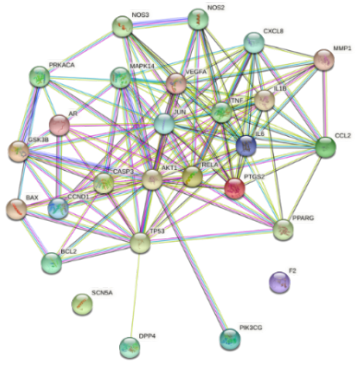

Figure 3: PPI Network diagram of intersecting targets of Atractylodes Rhizoma and COVID-19

3.3 GO enrichment analysis and KEGG pathway analysis

A total of 297 items ($P < 0.05$) were obtained from GO function enrichment, including 241 items of biological process (BP), 18 items of cell composition (CC) and 38 items of molecular function (MF). The targets mainly focus on such biological processes as positive regulation of gene expression, inflammatory response, cell proliferation and apoptosis, as shown in Figure 4. The enrichment of KEGG pathway resulted in 98 signal pathways ($P < 0.05$), mainly involving human cytomegalovirus infection, IL-17 signal pathway, TNF signal pathway, etc., as shown in Figure 5.

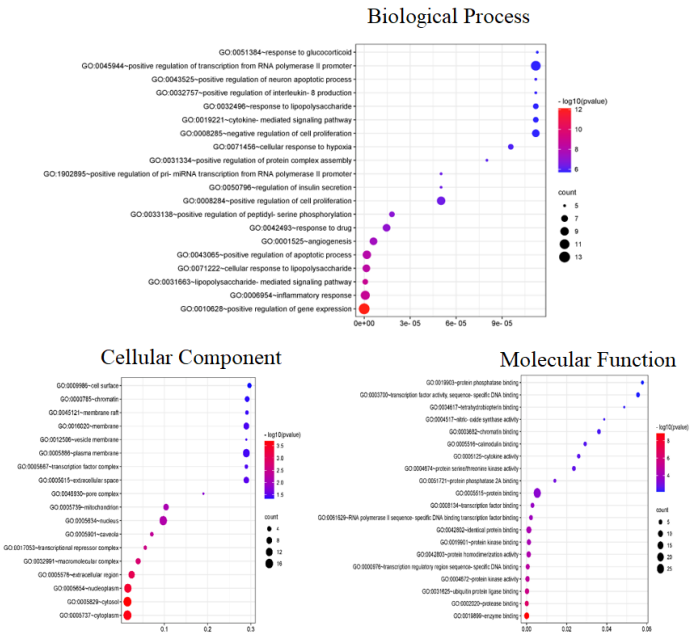

Figure 4: GO enrichment bubble Chart of Atractylodes Rhizoma in treating COVID-19

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Figure 5: KEGG pathway enrichment bubble Chart of Atractylodes Rhizoma in treating COVID-19

3.4 "Disease-pathway-target-component-drug" network

Construct the "disease-pathway-Target-Component-Drug" network diagram using Cytoscape 3.9.1. There are 7 active components of Atractylodes Rhizoma with corresponding targets. Each active compound can act on multiple targets, and multiple targets correspond to 20 signal pathways, which reflects the characteristics of multi-component, multi-target and multi-pathway interaction.

The results are shown in Figure 6. Red represents Atractylodes Rhizoma and COVID-19, pink represents interacting targets, blue represents active components of Atractylodes Rhizoma, green represents COVID-19 disease signaling pathways, lines represent Atractylodes Rhizoma and active components, active components and targets, COVID-19 and signaling pathways, and interactions between signaling pathways and targets.

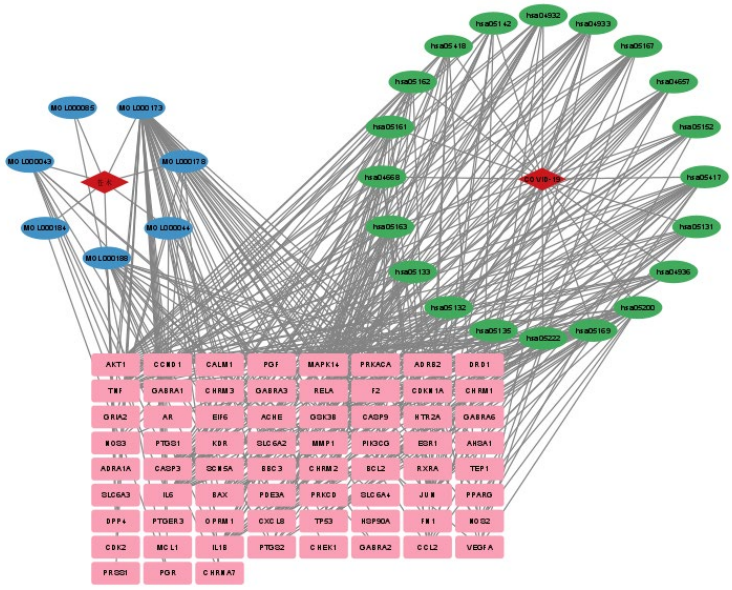

Figure 6: The "disease-pathway-target-ingredient-drug" network of Atractylodes Rhizoma in treatmenting COVID-19

3.5 Molecular docking results of active ingredients in Atractylodes Rhizoma acting on COVID-19

Through Autodock analysis, Get the results of the active components of Atractylodes Rhizoma with the COVID-19 related protein receptor. It is generally believed that the lower the energy is, the greater the possibility of action is when the conformation of ligand and receptor is stable[14]. Here, we select binding free energy ≤−5 Kcal/mol as the screening criteria [15]. The results showed that the active ingredients screened in Atractylodes Rhizoma could bind to ACE2, SARS-CoV-2 3CL and SARS-CoV-2 3CLpro protein receptors, and the binding free energy was shown in Table 2. See Figure 7 and Figure

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8 for molecular docking mode diagram and two-dimensional interaction diagram.

Table 2: Results of docking between active ingredients and receptor proteins in Atractylodes Rhizoma

Mol ID	Compound name	ACE2 binding energy (kcal/mol)	3CL binding energy (kcal/mol)	3CLPro binding energy (kcal/mol)
MOL000173	wogonin	-6.18	-6.34	-7.02
MOL000179	2-Hydroxyisoxypropyl-3-hydroxy-7-isopentene-2,3-dihydrobenzofuran-5-carboxylic	-5.7	-4.94	-6.08
MOL000184	NSC63551	-8.64	-7.98	-8.58
MOL000186	Stigmasterol 3-O-beta-D-glucopyranoside_qt	-8.48	-6.38	-8.47
MOL000188	3β-acetoxyatractylone	-7.17	-5.67	-6.85
MOL000085	beta-daucosterol_qt	-8.12	-6.01	-8.36
MOL000088	beta-sitosterol 3-O-glucoside_qt	-8.58	-7.03	-7.04
MOL000092	daucosterin_qt	-7.74	-6.49	-8.31
MOL000094	daucosterol_qt	-7.50	-6.73	-7.56

The results showed that the binding free energies of the active ingredients from Atractylodes Rhizoma with ACE2, SARS-CoV-2 3CL, and SARS-CoV-2 3CLpro protein receptors were less than −5.0 kcal/mol. MOL000184-NSC63551, MOL000186-Stigmasterol 3-O-beta-D-glucopyranoside_qt and MOL000088-beta-sitosterol 3-O-glucoside_qt combined well with ACE2. The good combination with SARS-CoV-2 3CL were MOL000184-NSC63551 and MOL000088- beta-sitosterol 3-O-glucoside_qt. The good combination with SARS CoV-2 3CLpro were MOL000184-NSC63551 and MOL000186-Stigmasterol 3-O-beta-D-glucopyranoside_qt. These results indicated that the active constituents of Atractylodes Rhizoma had good binding activity to ACE2, SARS-CoV-2 3CL and SARS-CoV-2 3CLpro.

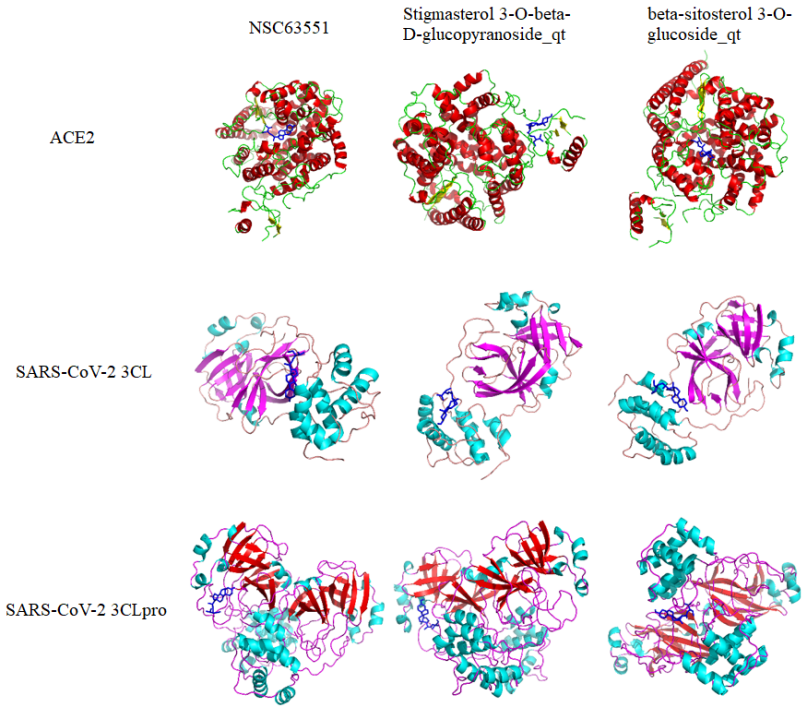

Figure 7: Molecular docking mode of receptor protein and some active components in Atractylodes Rhizoma

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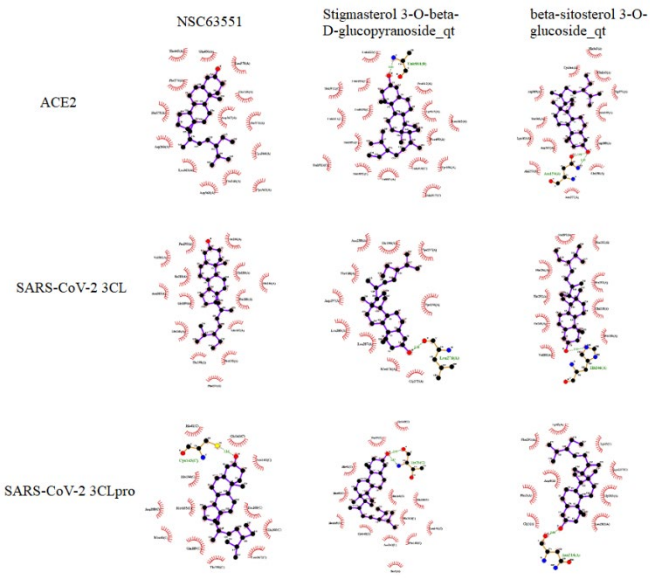

Figure 8: Two-dimensional diagram of the interaction between receptor proteins and some active components in Atractylodes Rhizoma

4. Discussion

2019-nCoV, which causes the novel coronavirus pneumonia COVID-19), is the seventh known coronavirus that can infect humans. It is a highly pathogenic β-coronavirus, which can cause acute respiratory infections and even death. Although it has been around for nearly three years, it is still ravaging the world, and there is still a lack of safe and effective drugs. Chinese herbal medicine has shown unique advantages in epidemic prevention and antiviral treatment, but its effective substances and mechanism of action have not been fully revealed [16]. COVID-19 (SARS-CoV-2) expresses 29 proteins, 16 of which are related to its replication. These proteins are generally targets of traditional small molecule drugs [17]. The highly conserved main protease (Mpro, also known as 3CLpro) in coronavirus is an important cysteine hydrolase, which plays a key role in the process of virus replication and is a key target for the prevention and treatment of coronavirus related diseases (including COVID-19) [18].

In this study, network pharmacology and molecular docking methods were used to explore the anti-COVID-19 mechanism of Atractylodes Rhizoma, and a "disease-pathway-target-component-drug" network was built for Atractylodes Rhizoma treatment of COVID-19. Nine active components were screened from Atractylodes Rhizoma, and 67 potential targets were obtained, 27 targets intersected with COVID-19. Including NOS2(nitric oxide synthase), AR(androgen receptor), SCN5A(sodium channel protein type 5 subunit α), PPARG(peroxisome proliferator-activated receptor γ), IL-6(interleukin-6) and DPP4(dipeptidyl peptidase IV), etc. This indicates that Atractylodes Rhizoma has the characteristics of multi-component, multi-target and multi-pathway treatment for COVID-19. The GO enrichment results show that the target mainly focuses on the positive regulation of gene expression, inflammatory response and other biological processes, and the enrichment of KEGG pathway mainly involves human cytomegalovirus infection, IL-17 signaling pathway, TNF signaling pathway, etc. These biological processes and signal pathways may play a key role in the treatment of COVID-19 with Atractylodes Rhizoma.

Based on previous results, this study selected several major proteins in COVID-19, (ACE2、SARS-CoV-2 3CL and SARS-CoV-2 3CLpro). Using molecular docking technology, The active components in Atractylodes Rhizoma were docked with them respectively, and their binding energies were determined. The results showed that the active ingredients in Atractylodes Rhizoma had good binding activity to ACE2, SARS-CoV-2 3CL and SARS-CoV-2 3CLpro. It indicates that Atractylodes Rhizoma has potential anti COVID-19 activity. There are some differences in amino acid fragments that bind various components of Atractylodes Rhizoma to protein receptors. For example, the binding model of NSC63551 and SARS CoV-2 3CLpro is composed of Pro293, Ile200, Gly109, Glu240, Thr198, Phe134, Pro132, Gln107, Pro108, Gln110, Ile249, Val202, Asn203, His246 amino acids; The binding model of Stigmasterol 3-O-beta-D-glucopyranoside and SARS CoV-2 3CLpro is composed of Thr199, Asn238,

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Thr198, Asp197, Leu286, Leu287, Met276, Gly275, Leu271, Tyr239, Tyr237 amino acids. This confirms the multi-component and multi-target role of Atractylodes Rhizoma against COVID-19.

To sum up, this study can quickly and efficiently screen out the potential active ingredients of Atractylodes Rhizoma against COVID-19, such as wogonin, NSC63551, Stigmasterol 3-O-beta-D-glucopyranoside. It acts on NOS2, AR, SCN5A, PPARG, IL-6, DPP4 and other targets through multiple pathways such as human cytomegalovirus infection, IL-17 signaling pathway, and TNF signaling pathway, thus playing the role of anti COVID-19. In view of the limitations of network pharmacology and molecular docking, the main active ingredients, key targets and pathways of Atractylodes Rhizoma should be verified in the later stage, In order to provide basis for Atractylodes Rhizoma to treat COVID-19.

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