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# Network pharmacological approach for elucidating the mechanisms of traditional Chinese medicine in treating COVID-19 patients

Despite advances in understanding the pathogenesis of 2019 novel coronavirus disease (COVID-19), there are yet no licensed vaccine or specific antiviral medicine to prevent or treat COVID-19 to date. Integrated multi-disciplinary treatment is recommended to improve therapeutic outcomes. To treat COVID-19 patients in China, Traditional Chinese Medicine (TCM) experts have recommended the use of herbal formulae based on their clinical expertise, and more than 90 % confirmed cases have received TCM therapy [1]. One of the TCM formulae was reported to be used in treating 701 confirmed cases across 10 provinces in China, of which most patients were relieved [2]. In addition, several ongoing randomized, clinical trials of TCM remedies have been registered in the international clinical trials registry platform (www.chictr.org.cn). However, these current data are insufficient to support the effectiveness and safety of TCM formulae in treating COVID-19 patients, the underlying mechanisms of TCM formula on blocking COVID-19 remain unclear. Computer-aided systematic approaches hold the promise of expanding understanding the rationale and mechanism for the clinical performance of TCM against COVID-19. In this study, we focused on the most widely used Chinese medicinal herbs contained in the prescribed formulae in treating COVID-19 and employed network pharmacology-based technologies to analyze and identify their underlying mechanisms from a holistic perspective.

To better interpret the scientific outlook on TCM, we collected all the clinically effective formulae that are included in the National Guidelines, Provincial Recommendations or Registered Clinical Trials (www.chictr.org.cn) up to February 23th. A total of 167 TCM formulae with 255 differentiated herbs, 22 mineral medicines and 22 animal parts were used in China (Supplementary table 1). Of note, 10 Chinese herbs, i.e. *Glycyrrhizae Radix Et Rhizoma, Armeniacae Semen Amarum, Gypsum Fibrosum, Scutellariae Radix, Forsythiae Fructus, Poria, Ephedrae Herba, Citri Reticulatae Pericarpium, Pogostemonis Herba, Lonicerae Japonicae Flos*, were applied in quite a high frequency, listed in at least 30 formulae. We have therefore defined these 10 herbs as a new formula called “Anti-COVID-19 Decoction” and analyzed their active compounds by adopting Traditional Chinese Medicine for Systems Pharmacology Database (TCMSP) (http://lsp.nwu.edu.cn/index.php), with absorption, distribution, metabolism and excretion (ADME) screening (oral bioavailability ≥30 % and drug-likeness value≥ 0.18), and Traditional Chinese Medicine Integrative Database (TCMID) (http://www.megabionet.org/tcmid/). As a result, a total of 258 compounds were identified as the bioactive ones (Supplementary table 2, 3), among which 20 compounds were contained in at least two herbs while the flavonoid quercetin was identified in 5 herbs of the Anti-COVID-19 Decoction, suggesting that functional coordination in some related biological processes may exist in the Decoction (Supplementary table 4).

Interestingly, we got 3215 compound-targets (with duplications) of the Anti-COVID-19 Decoction from TCMSP (Supplementary table 5), while 913 disease targets from GeneCards database (https://www.genecards.org/) and OMIM database (http://omim.org/) by adopting the keyword “new coronavirus” (Supplementary table 6). Of note, fifty-three overlapping genes were identified as the compound-targets of the Anti-COVID-19 Decoction by matching to the disease targets of COVID-19 (Supplementary Fig. 1), indicating that these 53 targets were the potential therapeutic targets of the Anti-COVID-19 Decoction against COVID-19. The Chinese herbal medicine of *Glycyrrhizae Radix Et Rhizoma* affected 46 COVID-19-associated disease targets, higher than other herbs in the Decoction (Supplementary Fig. 2). By conducting compound-target-disease network analysis, we found close correlations among the compounds in the Anti-COVID-19 Decoction and COVID-19-associated disease targets, indicating that multi-COmpounds contained in the Anti-COVID-19 Decoction might play their therapeutic roles through multiple COVID-19-associated targets (Supplementary Fig. 2). In particular, prostaglandin G/H synthase 2 (PTGS2), alpha-1D adrenergic receptor (ADRA1D), prostaglandin G/H synthase 1(PTGS1), nuclear receptor coactivator 2 (NCOA2) and peroxisome proliferator-activated receptor gamma (PPAR-γ) were targeted by 176, 151, 118, 112 and 102 compounds, respectively, indicating the potential synergistic effects in this herbal decoction.

We further performed the protein-protein interaction (PPI) analysis on those 53 potential therapeutic targets (https://string-db.org/), and found that interleukin 6 (IL-6) and mitogen-activated protein kinase3 (MAPK3) were the core targets of the Anti-COVID-19 Decoction (Fig. 1, Supplementary figure 4). The expression level of IL-6 was significantly increased in the severe COVID-19 patients compared to the level in mild cases [3]; while IL-6 receptor inhibitor tocilizumab has been applied in the patients to counteract the cytokine storm of COVID-19. Hence, inhibition of the expression level of IL-6 could be a promising therapeutic strategy for treating COVID-19. In this current study, we selected cytokine IL-6 as the major COVID-19-associated disease target for further investigation. We found that four compounds, i.e. quercetin, wogonin, luteolin and oroxylin a, were closely correlated with IL-6. The action network of these four compounds on the COVID-19-associated disease targets have been simultaneously identified (Supplementary figure 5). MAPK signaling pathway involves a wide range of cellular functions like cell proliferation, differentiation and survival, which plays crucial roles in coronavirus propagation. Some compounds contained in “Anti COVID-19 Decoction”, such as naringenin, have been previously proven to significantly inhibit on activated MAPK3, supporting the results of the network analysis partly [3,4].

To uncover the underlying mechanisms of the Anti-COVID-19 Decoction, we conducted functional analysis on the 53 potential therapeutic targets by gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) analysis. The results of GO analysis mainly focused on cytokine receptor binding, cytokine activity and receptor-ligand activity (Supplementary figure 6), while the results of KEGG

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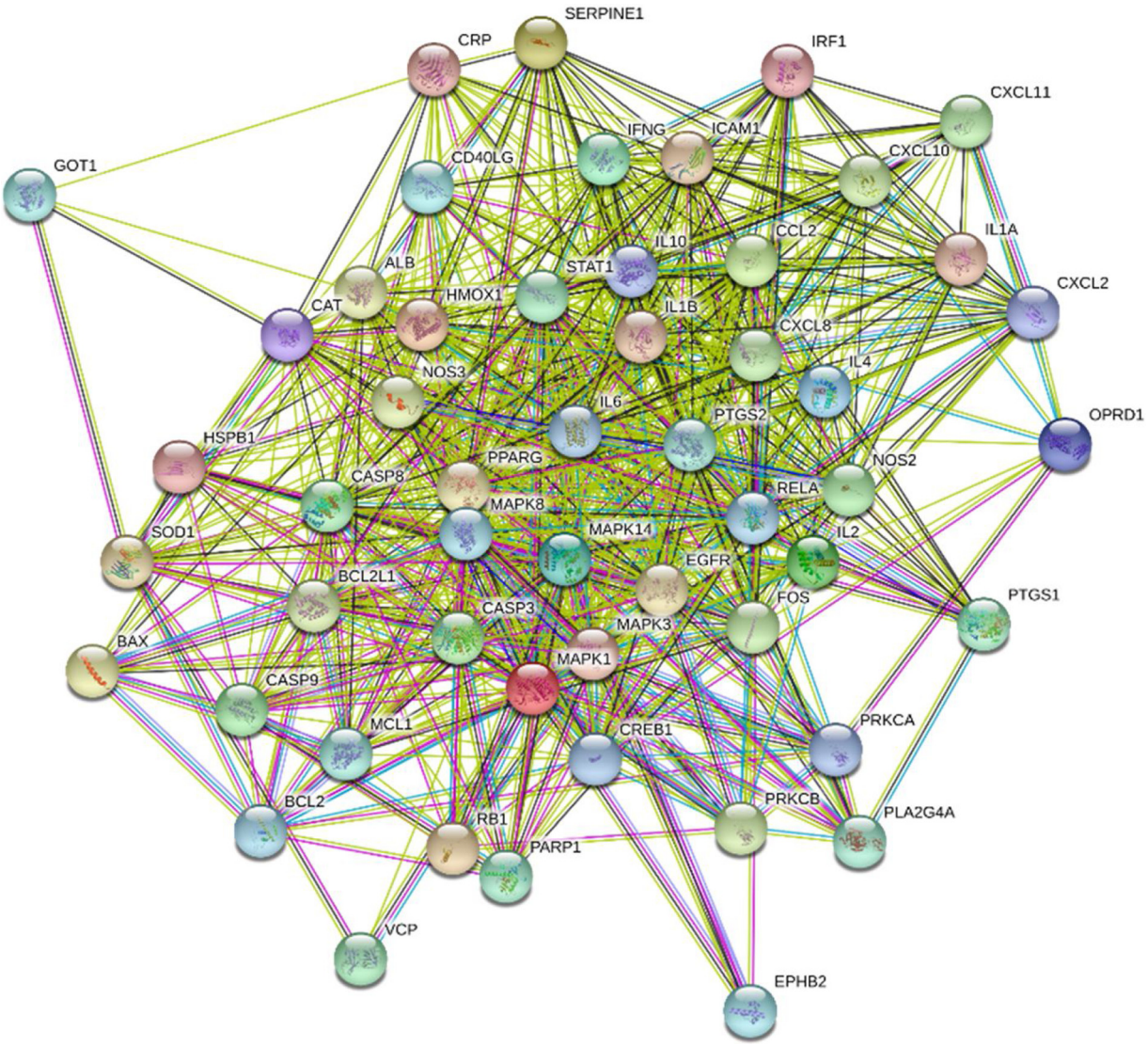


**Fig. 1.** PPI analysis on the potential therapeutic targets of the Anti-COVID-19 Decoction. Network nodes represent proteins. Edges represent protein-protein associations (Blue line: Known interactions from curated databases; fuchsia line: experimentally determined interactions; green line: gene neighborhood; red line: gene fusions; blue line: gene co-occurrence; yellow line: textmining; black line: co-expression; violet line: protein homology).

analysis revealed that the potential therapeutic mechanisms of the Anti-COVID-19 Decoction are most likely related to IL-17 signaling pathway and TNF signaling pathway (Supplementary figure 7&8, Supplementary table 7). Though the counts of peripheral T cells were substantially reduced, their status was hyperactivated and there was an increased concentration of the highly pro-inflammatory $CCR4^+CCR6^+$ Th17 in severe case [5]; moreover, the patients in ICU had a higher concentration of TNFα than those not requiring ICU admission [1], indicating that Th17 and TNFα account for the severe immune injury in the patients in part. Thus, modulation of IL-17 and TNFα signaling pathways by the Anti-COVID-19 Decoction may have significant potential to block the conversion of mild cases to severe cases, and even to save the critically ill patients.

Computed tomography imaging of COVID-19 patients is characterized by ground-glass opacity (56.4 %) and bilateral patchy shadowing (51.8 %) with partial consolidation which will be absorbed with the formation of fibrotic stripes [6,7]. Histological examination of the biopsy lung sample from the COVID-19 patient showed bilateral diffuse alveolar damage with cellular fibromyxoid exudates [5]. Therefore, the treatment for COVID-19 needs to deliver one-two punch to block the progression of pneumonia as well as to prevent the formation of fibrosis. Previous reports showed that inhibition of transforming growth factor-β (TGF-β) has the capability of preventing lung fibrosis [8]. We further applied molecular docking to calculate the possible interactions between the key compounds (quercetin, wogonin, luteolin and oroxylin a) and TGF-β. Binding free energies of quercetin, wogonin, luteolin and oroxylin a with TGF-β were -10.97 kcal $mol^{-1}$, -7.878 kcal $mol^{-1}$, -10.319 kcal $mol^{-1}$ and -8.408 kcal $mol^{-1}$, respectively (Fig. 2), indicating that these compounds might bind with TGF-β.

In conclusion, it was the first report focusing on the most widely used herbs in treating COVID-19 patients, which encourages us to identify the most effective anti-COVID-19 decoction from Chinese herbal medicine. And, multi-COmpounds in the Anti-COVID-19 Decoction are proposed to play a synergistic and multiple effects on the multiple COVID-19-associated disease targets, especially on modulating the disordered virally driven hyperinflammation. These results indicate that this formula may play important roles on the different stages of the disease, rather than a single stage or TCM syndrome of the COVID-19 patients. The key compounds in the Anti-COVID-19 Decoction are quercetin, wogonin, luteolin and oroxylin a; and these four compounds might have potentials of further development as new anti-COVID-19 agents. We anticipate that comprehensive pharmacological experiments

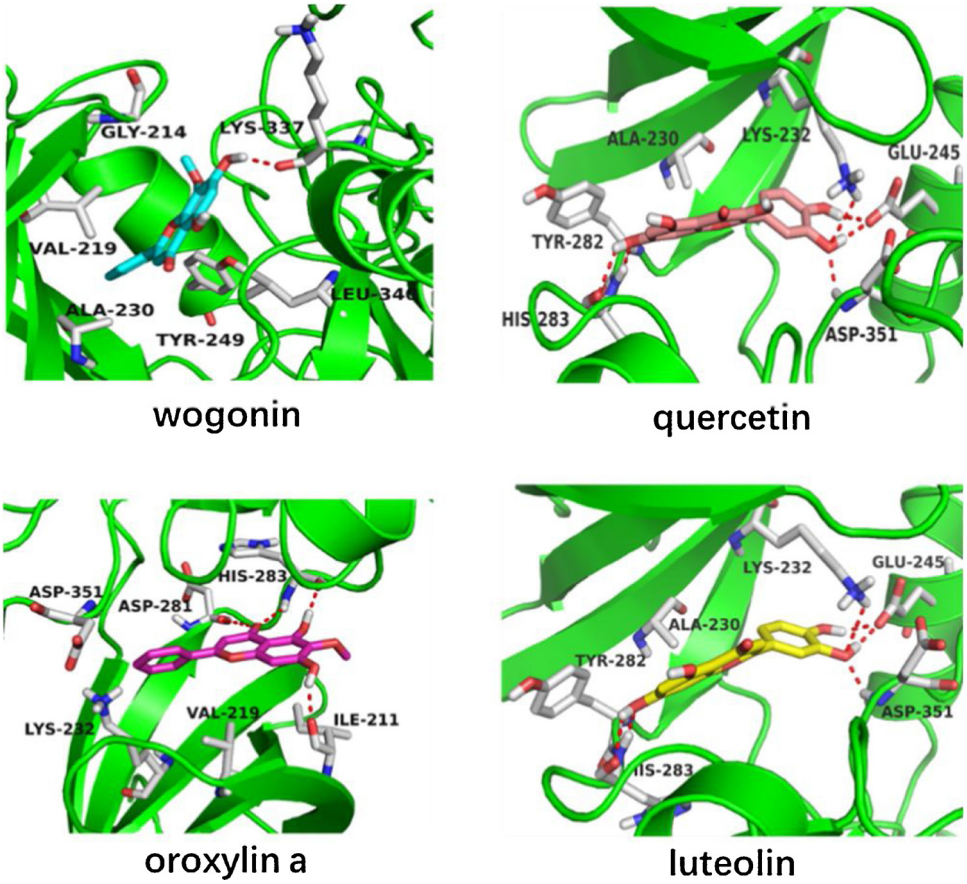


**Fig. 2.** The binding mode between the key compounds in the Anti-COVID-19 Decoction and TGF-β calculated by molecular docking.

*in vivo* and *in vitro* are needed to verify the therapeutic mechanisms of the Anti-COVID-19 Decoction in TCM. At the same time, well-designed clinical trials are also required to develop world-wide acceptance of TCM in treating COVID-19 patients.

## Authors' contributions

Study design, manuscript revision and funding acquisition, L Liu; Data analysis and writing, HD Pan; Molecular docking calculation, XJ Yao; Data collection, WY Wang; Manuscript revision, HY Lau.

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## Declaration of Competing Interest

The authors have declared that there is no conflict of interest.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phrs.2020.105043.

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