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Network pharmacology and computational analysis of berberine and kuwanon Z as possible natural antiviral compounds in COVID-19

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Author contributions

All the authors have carefully reviewed and approved the final version of the manuscript. All the authors contributed equally.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

RMSD, root mean square deviation; Mpro, main protease; COVID-19, coronavirus disease 19; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; BBR, Berberine.

Citation

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Abstract

Background: Global efforts to discover effective therapeutic agents for combating coronavirus disease 19 (COVID-19) have intensified the exploration of natural compounds with potential antiviral properties. In this study, we utilized network pharmacology and computational analysis to investigate the antiviral effects of Berberine and Kuwanon Z against severe acute respiratory syndrome coronavirus 2, the viruses responsible for COVID-19. **Method:** Utilizing comprehensive network pharmacology approaches, we elucidated the complex interactions between these compounds and the host biological system, highlighting their multitarget mechanisms. Network pharmacology identifies COVID-19 targets and compounds through integrated protein–protein interaction and KEGG pathway analyses. Molecular docking simulation studies were performed to assess the binding affinities and structural interactions of Berberine and Kuwanon Z with key viral proteins, shedding light on their potential inhibitory effects on viral replication and entry. **Results:** Network-based analyses revealed the modulation of crucial pathways involved in the host antiviral response. Compound-target network analysis revealed complex interactions (122 nodes, 121 edges), with significant interactions and an average node degree of 1.37. KEGG analysis revealed pathways such as the COVID-19 pathway, chemokines and Jak-sat in COVID-19. Docking studies revealed that Kuwanon Z had binding energies of −10.5 kcal/mol for JAK2 and −8.1 kcal/mol for the main protease. **Conclusion:** The findings of this study contribute to the understanding of the pharmacological actions of Berberine and Kuwanon Z in the context of COVID-19, providing a basis for further experimental validation. These natural compounds exhibit promise as potential antiviral agents, offering a foundation for the development of novel therapeutic strategies in the ongoing battle against the global pandemic.

Keywords: COVID-19; kuwanon Z; berberine; network pharmacology; SARS-CoV-2; molecular docking

Background

The global impact of the coronavirus disease 19 (COVID-19) pandemic, attributed to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has significantly affected public health, economies, and daily routines since its onset in late 2019. Originating in Wuhan, China, the virus rapidly disseminated across the globe, prompting the *World Health Organization* to declare it a pandemic on March 11, 2020 [1]. According to the most recent data available, COVID-19 has resulted in millions of confirmed cases and substantial mortality across diverse geographical regions. SARS-CoV-2, a novel beta coronavirus, is characterized by its high transmission efficiency through respiratory droplets and direct human-to-human contact.

The clinical presentations of COVID-19 span a spectrum, encompassing asymptomatic or mild respiratory symptoms to severe pneumonia, acute respiratory distress syndrome, and in certain instances, fatality. In addition to respiratory involvement, COVID-19 has been associated with multiple organ manifestations, affecting the cardiovascular, renal, hepatic, and gastrointestinal systems [2]. Scientific efforts have been dedicated to understanding the virology, pathogenesis, and clinical characteristics of SARS-CoV-2. The virus genome was swiftly isolated and sequenced in early 2020, providing valuable insights into its genetic makeup [3]. While significant strides have been made in developing and administering COVID-19 vaccines, challenges remain, including vaccine distribution, accessibility, and addressing emerging variants of the virus. Additionally, the global community faces ongoing dilemmas related to vaccination strategies, the long-term effectiveness of vaccines, and the need for alternative therapeutic approaches.

During SARS-CoV-2 infection, the innate immune response may be inhibited or delayed, potentially leading to persistent viral replication. This, in turn, can trigger emergency signals that contribute to a cytokine storm, a phenomenon associated with the severe progression of COVID-19. Cytokines play a pivotal role in regulating the immune response and facilitating virus clearance, making them crucial components in the altered response to SARS-CoV-2. Cytokines exert their effects through a family of more than 40 transmembrane receptors that are associated with one or more of the four JAK2 encoded by the human genome—namely, JAK1, JAK2, JAK3, and TYK2. Upon activation, JAK2 influences pathways related to survival, proliferation, differentiation, and immune regulation and, in the case of type I interferons, has antiviral and antiproliferative effects. Cytokines function through specific receptors, more than 40 of which are encoded in the human genome. These receptors signal via JAK2, originally named Just another Kinases, which become noncovalently attached to their cytosolic tails.

Among the multitude of signaling pathways involved in the host's reaction to viral infections, the JAK2 signaling pathway is a crucial contributor to the inflammatory cascade linked to COVID-19. The JAK2 signaling pathway is important for facilitating cellular responses to cytokines and growth factors, influencing immune cell activation, proliferation, and differentiation. Recent studies have highlighted the dysregulation of the JAK2 pathway in the context of COVID-19, indicating its potential as a therapeutic target. Several investigations have demonstrated the upregulation of JAK2 and associated downstream signaling molecules in response to SARS-CoV-2 infection. Dysregulation of JAK2 signaling has been associated with the cytokine storm observed in severe cases of COVID-19, which is characterized by an excessive release of proinflammatory cytokines and chemokines. Prolonged activation of the JAK2 pathway contributes to the hyperinflammatory state, resulting in tissue damage and organ dysfunction. In addition to its involvement in inflammation, the JAK2 signaling pathway is implicated in the regulation of thrombosis, a prominent aspect of severe COVID-19. Anomalies in JAK2 activation, including pulmonary embolism and disseminated intravascular coagulation, have been linked to an elevated risk of thrombotic events. This dual role of JAK2 in inflammation and thrombosis underscores its significance as a potential target for therapeutic intervention in patients with COVID-19 [4].

Coronaviruses, which belong to the RNA virus class, exhibit a single-stranded positive-sense genome and are prevalent in both wildlife and humans. Notably, these viruses boast the largest RNA genomes among known viruses. Consequently, the virus encodes two overlapping open-reading frames, giving rise to two polyproteins: pp1a and pp1ab. The subsequent processing of these polyproteins leads to the formation of sixteen nonstructural proteins (nsps) and four structural proteins. The virus replicase polyprotein undergoes proteolytic processing facilitated by two distinct cysteine proteases: the papain-like protease (PLpro) and the main protease (Mpro). The proteolytic refinement of nsps by PLpro and 3Clpro is pivotal for virus maturation and replication, rendering these proteases crucial druggable targets [5].

In particular, the Mpro of SARS-CoV-2 has become a focal point of investigation due to its essential role in the viral life cycle and replication. Also known as the 3C-like protease (3Clpro), Main protease (Mpro) is indispensable for proteolytic processing and maturation of the virus, making it a promising target for antiviral drug development. During the early stages of the outbreak, scientists successfully isolated and sequenced the genome of SARS-CoV-2, leading to the identification of Mpro as a vital protein. Subsequent research efforts have delved into the structural and functional aspects of the main protease in COVID-19, providing valuable insights into its role in viral replication and pathogenesis. Notably, a study by Liu et al. presented the crystallized structure of the main protease of COVID-19, highlighting its potential as a therapeutic intervention target [6]. The efficacy of medicinal plants in treating a diverse range of infectious and noninfectious diseases has increased. Approximately 25% of commonly used pharmaceuticals are derived from plant-based compounds. In contemporary medical research, driven by advancements in separation techniques and the rise of emerging infectious diseases, plants and their bioactive constituents hold promise as potential sources for drug discovery.

Berberine (BBR), a quaternary ammonium derivative derived from isoquinoline salts, is a notable compound in this context. It is an odorless yellow powder with a bitter taste and thus high water solubility and slight solubility in methanol and ethanol. Berberine can be readily obtained either from medicinal plants or through total synthesis. Widely distributed in the roots, stems, and rhizomes of various plant families, such as Euphorbiaceae, Ranunculaceae, and Papaveraceae. BBR has emerged as a compelling subject for further exploration in the quest for novel therapeutic agents. In a recent investigation, the significant anti-inflammatory, antioxidant, and antiviral attributes of BBR were elucidated. The compound effectively attenuated the inflammatory response linked to acute lung injury by promoting the nuclear translocation and phosphorylation of nuclear factor erythroid 2–related factor 2. This action results in a reduction in inflammatory factor and reactive oxygen species production [7]. Berberine induces apoptosis in virus-infected cells by promoting the generation of reactive oxygen species [8, 9]. This dual mechanism, addressing both disease resistance and viral destruction, is notable and distinctive. Direct interaction of BBR with virions impedes infection and inhibits SARS-CoV-2 replication [10].

Moreover, Berberine has been shown to reduce the levels of circulating inflammatory mediators, including interleukin-6, tumor necrosis factor-α, and CRP, in individuals afflicted with severe COVID-19 [11]. Additionally, it hindered the proliferation of SARS-CoV-2-infected cells and alleviated associated inflammatory disorders by modulating inflammatory signaling pathways [12]. Flavonoids are plant-derived polyphenolic compounds with antioxidant properties. Among the flavonoids in this class of compounds, Kuwanones exhibit diverse biological activities. Mulberry plants contain various types of Kuwanons, including Kuwanon H, G, L, S, and T (isolated from root bark), Kuwanon X (extracted from leaves), and Kuwanon C (derived from roots). Additionally, Kuwanon Z, sourced from *Morus alba*, has been documented [13]. Numerous studies have explored the multifaceted biological effects of Kuwanon, including its anti-inflammatory, antioxidant, antibacterial, antiviral, and tyrosinase inhibitory effects [14]. These compounds have

garnered attention for their potential health-promoting effects, including anti-inflammatory, antiviral, and anticancer activities. In the past decade, an increasing number of active phytochemicals with therapeutic potential have been identified. The antiviral effects of these agents are attributed to scavenging capacities, antioxidant activities, or the inhibition of RNA/DNA replication. Furthermore, these compounds demonstrate low cytotoxicity and high bioavailability, rendering them attractive candidates for antiviral drug development. In light of this, the present study focused on investigating the potential antiviral effects of the phytochemicals Berberine and Kuwanon Z against COVID-19 utilizing network pharmacology and molecular docking studies.

Materials and methods

PPI network

PPI analysis constitutes a fundamental tool for elucidating the intricate involvement of proteins in diverse biochemical cascades, facilitating a comprehensive understanding of cellular architecture, biological processes, and functional modalities. This investigation utilized the sophisticated virtual screening platform STRING 11.0 (https://string-db.org/) to examine the complex web of interconnecting proteins [15]. The selected genetic components were meticulously uploaded to the STRING database, providing crucial insights into the multifaceted PPIs. The construction of the PPI network was meticulously tailored to the specific context of '*Homo* sapiens', and the criteria for evaluating the confidence level in the interactions among the target proteins were strictly calibrated to the highest degree of reliability, surpassing the threshold of data confidence set at 0.9. In this intricate network visualization, the individual network nodes were emblematic of distinct proteins, while the interlinking edges intricately depicted the associations among the diverse protein entities.

Selection of compound and disease target genes

To establish the compound-target network, an essential preliminary step involves the identification of disease-associated genes. To this end, pertinent information concerning the target genes associated with COVID-19 was meticulously gathered from authoritative sources such as the GeneCards (https://www.genecards.org) database. Additionally, comprehensive information regarding the protein targets of Berberine and Kuwanon Z was retrieved from SwissTargetPrediction (http://www.swisstargetprediction.ch/) [16].

Compound target network

After completion of the protein–protein interaction analysis, the intricate molecular mechanisms were elucidated via the construction of a comprehensive compound–target network via the Cytoscape visualization software v_3.7.1 [17]. This network architecture serves as a vital tool for comprehending and dissecting the intricate interplay between bioactive components and their corresponding targets and elucidating the underlying pathways implicated in this intricate biological network.

GO and KEGG pathway annotation

GO and KEGG pathway annotation were performed using the ShinyGo platform [18]. The purpose of the GO analysis was to systematically examine the gene cluster within the network, thereby refining the precision of the data prediction. GO encompasses a meticulously curated compilation of standardized terms pertaining to biological processes, molecular functions, and cellular components. These terms include both curated and predicted gene annotations derived from diverse species. The utilization of GO for annotating biological processes provides a robust resource for pathway enrichment analysis, aiding in the identification of crucial biological processes essential for obtaining meaningful functional insights aligned with the study objectives.

Furthermore, KEGG was used to investigate the functions and metabolic pathways associated with the network of genes and molecules under investigation. Its application extends to the elucidation of contributory pathways linked to disease phenotype, offering valuable insights into the intricate interplay between molecular entities and disease mechanisms. The utilization of these tools enhances the comprehensiveness and depth of our understanding of the biological processes and pathways relevant to the study.

Molecular docking

Software tools. The retrieval of target proteins involved the utilization of the Protein Data Bank (https://www.rcsb.org/). Subsequent to retrieval, the target structures were optimized, purified, and prepared for docking using Discovery Studio Visualizer 2020. This preparation included the elimination of undesired water molecules and bound ligands from the protein structure, followed by the saving of the optimized structures in the PDB file format within the same directory. Docking studies of selected ligands and approved drugs (molnupiravir, nirmatrelvir, and remdesivir) were conducted using AutoDock Vina 1.1.2 integrated into PyRx 0.8. The outcomes of these docking studies were subjected to analysis and visualization using PyMol software (The PyMOL Molecular Graphics System, Version 2.4.1 Schrödinger, LLC) [19].

Ligand preparation. The ligand and approved standard drug structures, provided in SDF format, were obtained from the U.S. National Library of Medicine PubChem official website (https://pubchem.ncbi.nlm.nih.gov/), as detailed in Table 1. These structures were subsequently imported into PyRx 0.8 utilizing the open Babel tool, and energy minimization (optimization) was conducted considering fundamental parameters such as element, hybridization, and connectivity based on the Universal Force Field. The ligand structures were subsequently converted to the AutoDock Ligand format (PDBQT).

Target preparation. Docking studies of selected natural compounds and approved standard drugs were performed using AutoDock Vina 1.1.2 in PyRx 0.8. The crystal structures of JAK2 and the main protease of COVID-19 complexed with the inhibitor N3 (PDB: 7F7 W and 6LU7, respectively) were employed for the analysis. The three-dimensional crystal structures of JAK2 (PDB: 7F7 W) and the main COVID-19 protease with the inhibitor N3 (PDB: 6LU7) were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/structure/7F7 W and https://www.rcsb.org/structure/6LU7, respectively), as outlined in Table 2 and depicted in Figure 1. For the docking procedure, the viral protein structure was optimized, purified, and prepared using Discovery Studio Visualizer 2020. This involved the removal of undesirable water molecules and bound ligands from the protein structure. The optimized structure was then saved in PDB file format within the same directory.

Docking procedure. The structures of the target proteins were input into the docking software PyRx 0.8 utilizing the "load molecule" option from the File toolbar. Subsequently, the receptor structure was converted into the AutoDock macromolecule format (PDBQT) using the right-click option. Binding affinity studies were conducted employing the Vina Wizard Tool in PyRx 0.8. The PDBQT files of both ligands and targets were chosen for the docking process. For the molecular docking simulations, a three-dimensional grid box was designed using the AutoDock tool 1.5.6. Specifically, a grid box of dimensions (size_x = −7.7274 Å; size_y = −41.1614 Å; size_z = −39.1428 Å) was established for JAK2 (PDB: 7F7 W), and another grid box with dimensions (size_x = −10.7292 Å; size_y = −12.4176 Å; size_z = 68.8161 Å) was created for the COVID-19 main protease (PDB: 6LU7), both with an exhaustiveness value of 8.

Following the selection of molecules, active amino acid residues were designated to define the cavity using the toggle selection spheres option provided in PyRx. The alignment of the grid box was adjusted to encompass all active binding sites and essential residues. Subsequently, ligands and targets were subjected to docking to assess their binding affinities. Initially, X-ray-bound native ligands were docked against the proteins, and root mean square deviation (RMSD) calculations were performed to validate the docking protocol. Once

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Table 1 2D and 3D structures of compounds with their ID

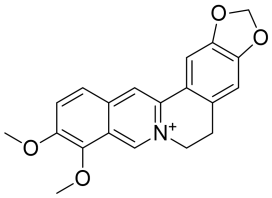
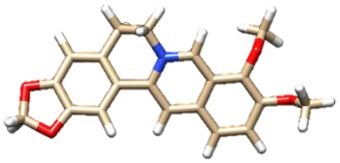
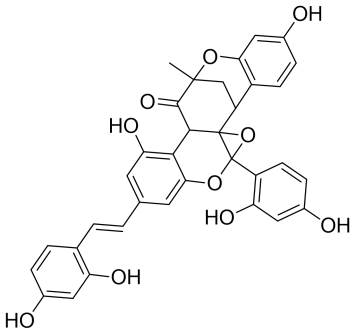
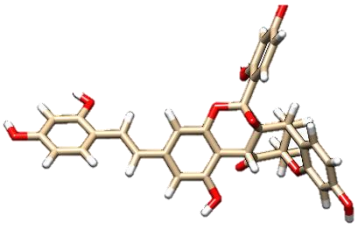
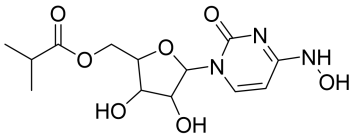
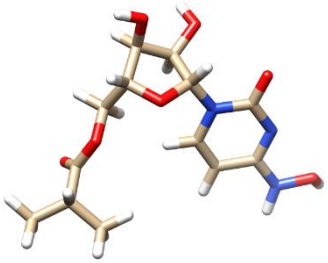
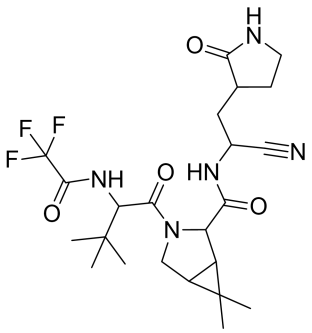
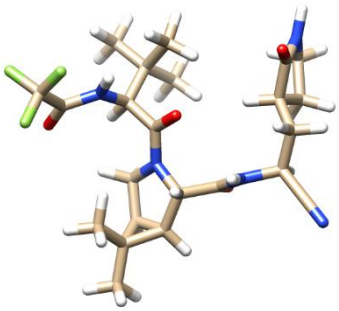
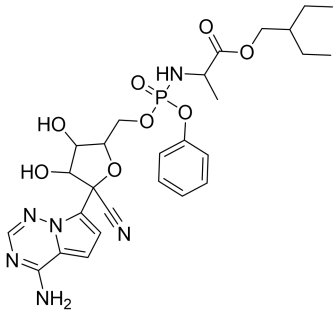
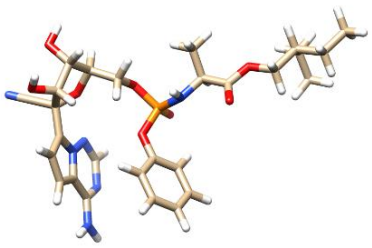
Compound	Drug ID	Chemical structure	
		2D	3D
Berberine	2353		
Kuwanon Z	21594954		
Molnupiravir	145996610		
Nirmatrelvir	55903259		
Remdesivir	121304016		

Table 2 The details of SARS-CoV-2 main protease (Mpro) used (PDB ID-6LU7) and Jak2 (Pdb Id: 7F7W)

PDB ID	JAK2 (PDB ID: 7F7W)	COVID-19 main protease (PDB: 6LU7)
Title	Jak2-Jh2	The crystal structure of COVID-19 main protease in complex with an inhibitor N3
Doi	https://Doi.Org/10.2210/Pdb7f7w/Pdb	https://Doi.Org/10.2210/Pdb6lu7/Pdb
Authors	Niu, L.	Liu X, Zhang B, Jin Z, Yang H, Rao Z.
Deposited On	2021-06-30	2020-01-26
Resolution	1.83 Å	2.16 Å
Classification	Transferase	Viral Protein
Organism (S)	Homo Sapiens	Severe Acute Respiratory Syndrome Coronavirus 2, Synthetic Construct
Expression System	Spodoptera Frugiperda	Escherichia Coli Bl21(De3)
Method	X-Ray Diffraction	X-Ray Diffraction

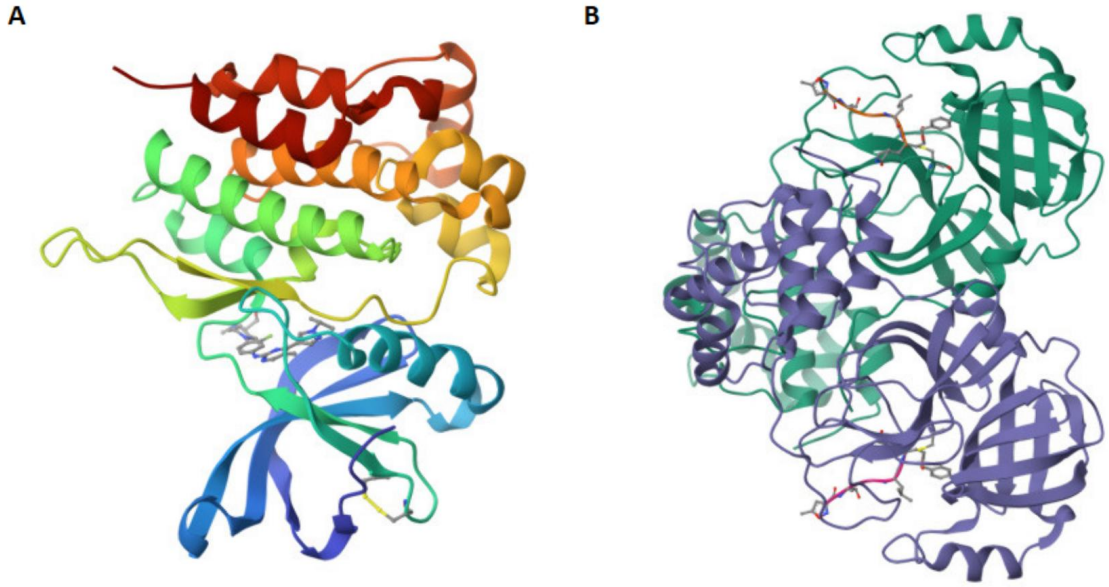


Figure 1 Protein targets. (A) JAK2 (PDB ID: 7F7W). (B) Main protease (PDB ID-6LU7).

validated, the same docking protocol was applied for subsequent docking with ligands.

Results

PPI network analysis

The target genes associated with the respective components were subjected to an in-depth analysis using STRING v_11 to construct and visualize the PPI network. High-confidence protein interaction data for a specific protein were subjected to filtration using a rigorous threshold established at a score surpassing 0.9. The resulting PPI network is depicted in Figure 2. There were 60 nodes and 41 edges, with each edge representing a distinct PPI. Notably, the average node degree, reflecting the number of connected targets within the network, was determined to be 1.37. A local clustering coefficient of 0.335 is indicative of the quantity of targets interlinked within the network. Analysis of the PPI network revealed the involvement of prominent targets, such as EGFR, SRC, HSP90AA1, HSP90AB1, PTGS2, CDC42, MAPK1, ICAM1, JAK2 and MAPK14, in COVID-19. Furthermore, the spatial distribution of EGFR, MET, JAK2, and SRC on the right of the network suggested their significant contribution to the pathogenesis of COVID-19.

Compoundtarget network

To explore the signaling pathways and functional consequences associated with the chosen target genes, the information was imported into Cytoscape, which enabled the creation of an extensive compound–target network. A network portraying interactions among compounds, targets, and diseases is depicted in Figure 3, which shows the intricate mechanisms underlying the pharmacological actions of the compounds in the context of COVID-19 treatment. The network included 2 distinct ingredients and revealed interactions with 121 target proteins. Notably, network analysis highlighted the convergence of multiple components on various targets, suggesting potential synergistic modulation by active biochemical entities. This intricate interplay may contribute to the therapeutic efficacy of these agents not only in COVID-19 management but also in the mitigation of other associated diseases and disorders. Comprehensive details of the network's topological parameters are provided in Supplementary Table S1, underscoring the pivotal role of each target within the complex network architecture. Topological parameters are essential in network pharmacology because they provide quantitative measures

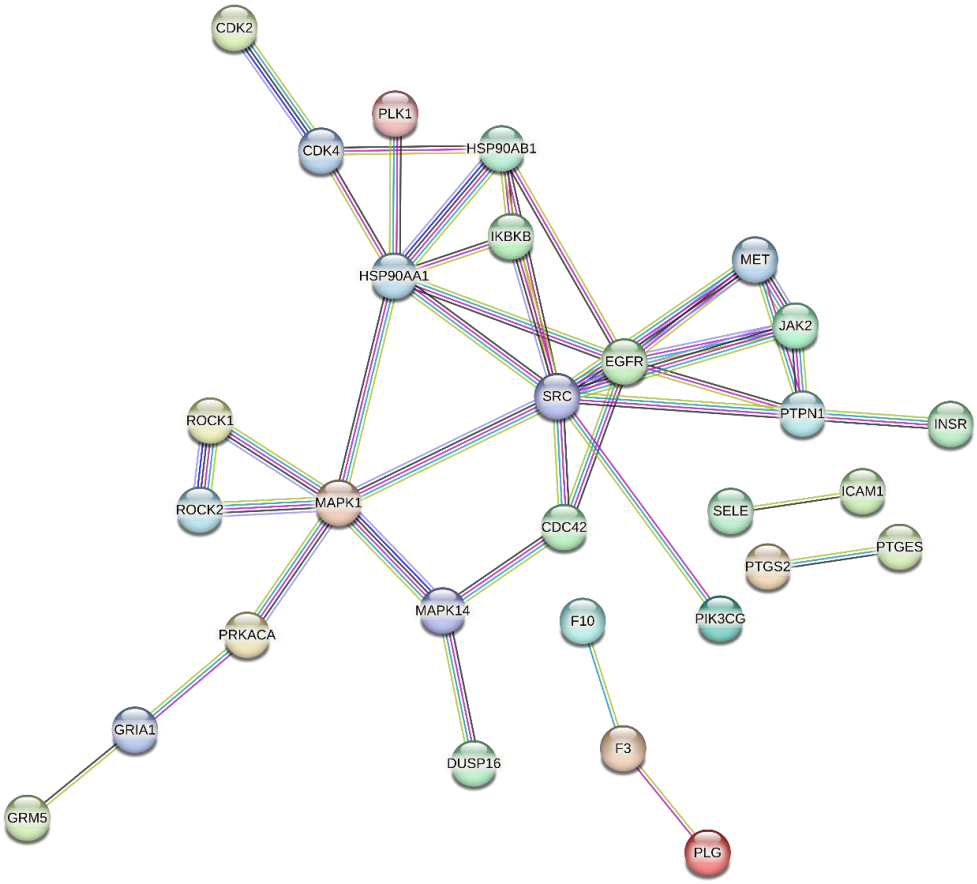


Figure 2 PPI network. Protein–protein interaction network of berberine and kuwanon Z in COVID-19.

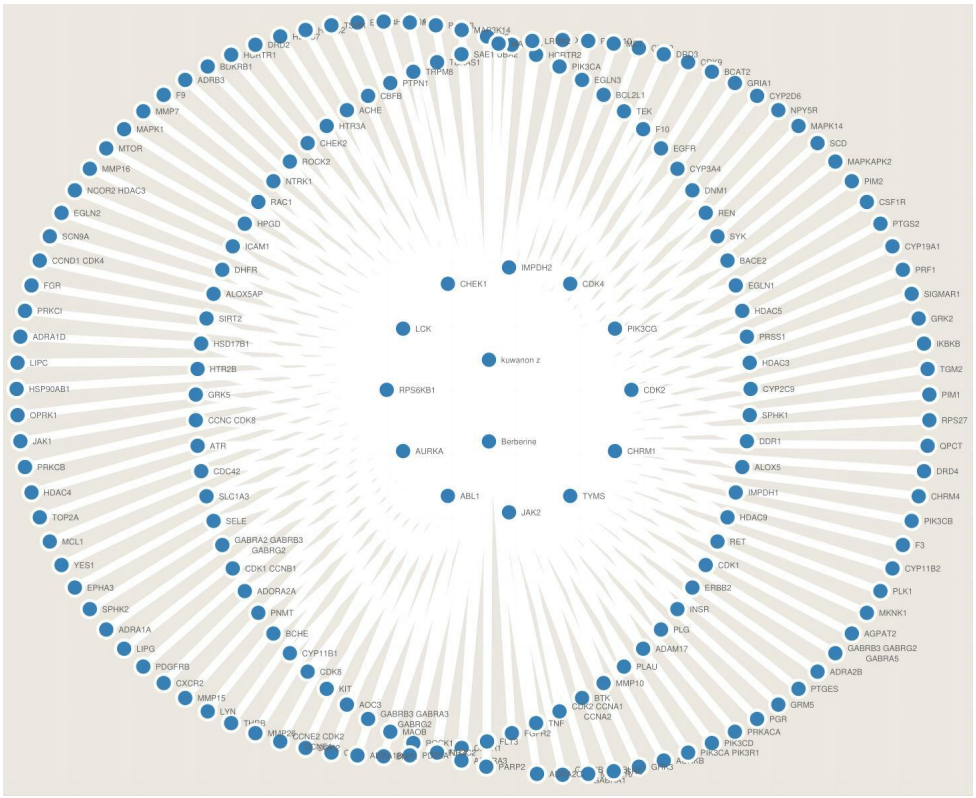


Figure 3 Compound target network

for characterizing and understanding the structure, function, and dynamics of complex biological networks. This information is valuable for identifying potential drug targets, unraveling disease mechanisms, and guiding therapeutic interventions.

GO pathway annotation

GO enrichment analysis was performed to investigate the underlying target proteins. The ShinyGO settings were configured using three criteria for the assessment of the target genes, focusing on GO biological (Table 3, Figure 4A), GO molecular (Table 4, Figure 4B), and GO cellular (Table 5, Figure 4C) processes, with a primary emphasis on the crucial KEGG pathway (Table 6, Figure 5).

Table 3 Go biological process

Description	Count in gene set	false discovery rate
Pos. reg. of phosphorylation	25	3.84×10^{18}
Pos. reg. of protein phosphorylation	22	6.80×10^{16}
Pos. reg. of phosphorus metabolic proc.	25	1.96×10^{17}
Response to nitrogen compound	26	4.23×10^{16}
Reg. of phosphorylation	27	3.44×10^{16}
Response to oxygen-containing compound	35	2.84×10^{21}
Protein phosphorylation	32	5.11×10^{19}
Pos. reg. of cell communication	31	3.68×10^{17}
Pos. reg. of signaling	31	3.68×10^{17}

Table 4 Go molecular process

Description	Count in gene set	False discovery rate
Transmembrane receptor protein tyrosine kinase activity	9	1.70×10^{9}
Protein kinase activity	23	5.37×10^{19}
Protein serine kinase activity	13	2.57×10^{10}
Protein serine/threonine kinase activity	15	1.55×10^{11}
Phosphotransferase activity, alcohol group as acceptor	23	1.01×10^{17}
Kinase activity	23	1.31×10^{16}
Transferase activity, transferring phosphorus-containing groups	23	4.95×10^{15}
ATP binding	25	1.52×10^{12}
Adenyl ribonucleotide binding	25	3.16×10^{12}
Adenyl nucleotide binding	25	3.21×10^{12}

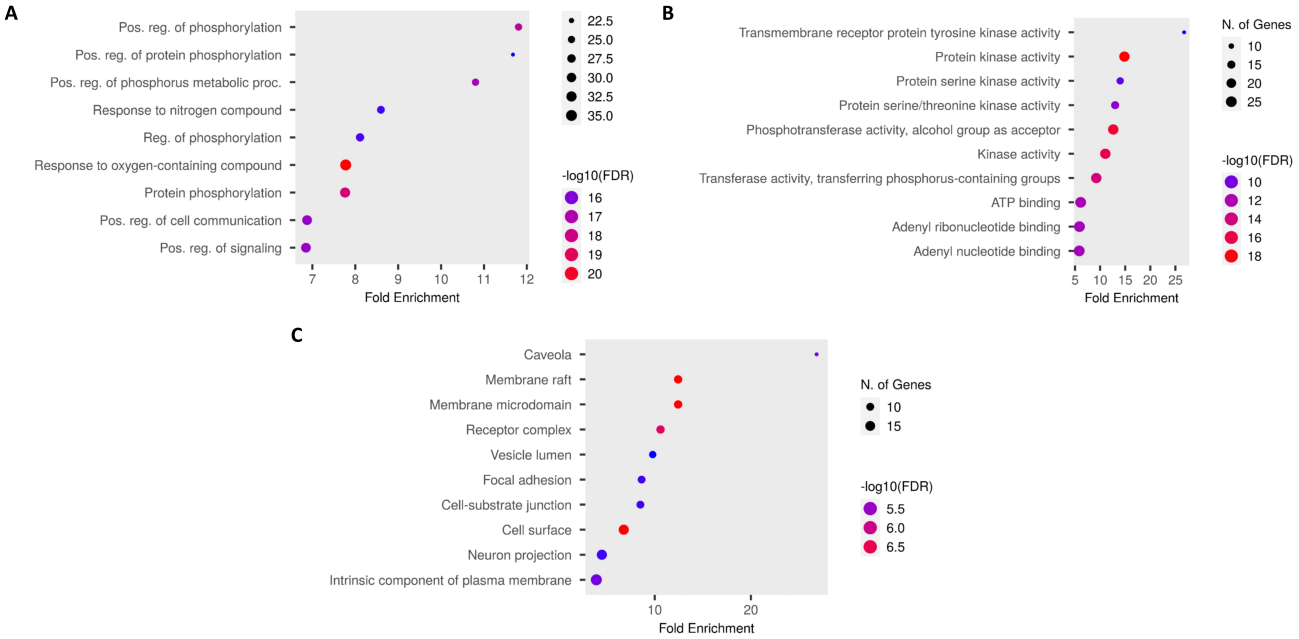


Figure 4 Enrichment analysis for gene ontology. (A) Biological process. (B) Molecular function. (C) Cellular component.

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Table 5 Go cellular components

Description	Count in gene set	False discovery rate
Caveola	6	5.34×10^{6}
Membrane raft	11	1.07×10^{7}
Membrane microdomain	11	1.07×10^{7}
Receptor complex	11	4.21×10^{7}
Vesicle lumen	9	9.34×10^{6}
Focal adhesion	10	7.93×10^{6}
Cell-substrate junction	10	7.93×10^{6}
Cell surface	16	1.07×10^{7}
Neuron projection	16	7.93×10^{6}
Intrinsic component of plasma membrane	19	5.34×10^{6}

Table 6 KEGG pathways

Description	Count in gene set	False discovery rate
Coronavirus disease	7	8.13×10^{6}
Chemokine signaling pathway	8	2.31×10^{7}
JAK-STAT signaling pathway	2	0.074624688
MAPK signaling pathway	11	4.04×10^{9}
IL-17 signaling pathway	7	4.84×10^{8}
Th17 cell differentiation	7	1.04×10^{7}
PI3K-Akt signaling pathway	14	8.77×10^{12}
Pathways in cancer	16	8.77×10^{12}
Lipid and atherosclerosis	12	8.77×10^{12}

Figure 5 KEGG pathways. Top 60 pathways.

The fusion of GO terms was restricted to a threshold of $P \leq 0.05$, as determined by the false discovery rate according to the Benjamini–Hochberg method. In light of this, comprehensive GO and KEGG analyses were performed to decipher the signaling pathways involved, revealing notable associations with the COVID-19 pathway (Figure 6), chemokines, Jak-sat, Th17 and interleukin-17 (Figure 7A–7B). Berberine and Kuwanon Z demonstrated potential applicability in the management of various conditions, including leishmaniasis, endocrine resistance, prostate cancer, Kaposi sarcoma-associated herpesvirus infection and human cytomegalovirus infection. While initially chosen for their examination of COVID-19-related effects, comprehensive KEGG analysis (Figure 5) revealed the involvement of these viruses in multiple disease pathways and disorders. Given these multifaceted interactions and the visualized network, the use of Berberine and Kuwanon Z as novel pharmacotherapeutic agents holds promise for the treatment of diverse diseases and disorders.

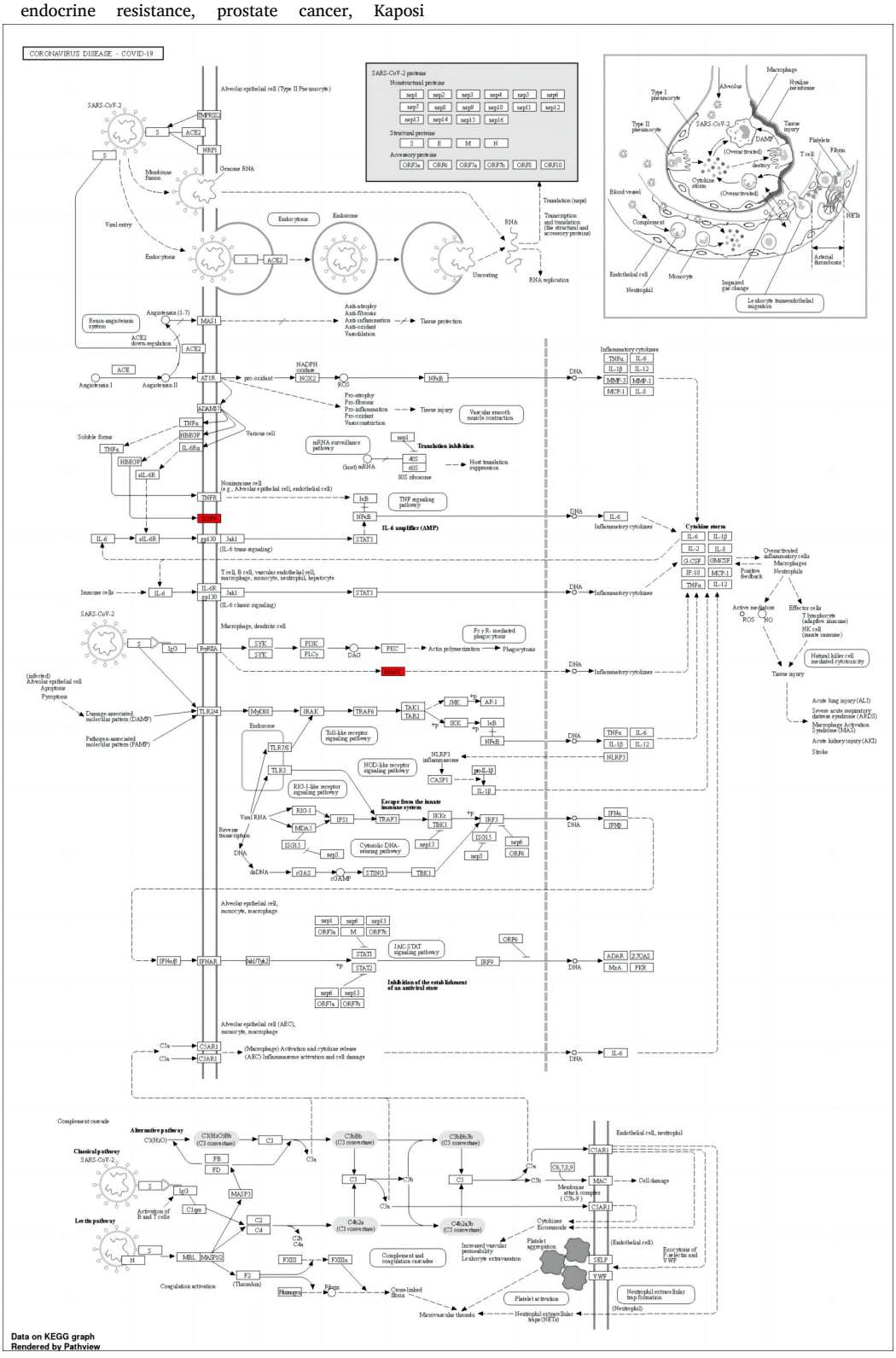

Figure 6 COVID-19 pathway

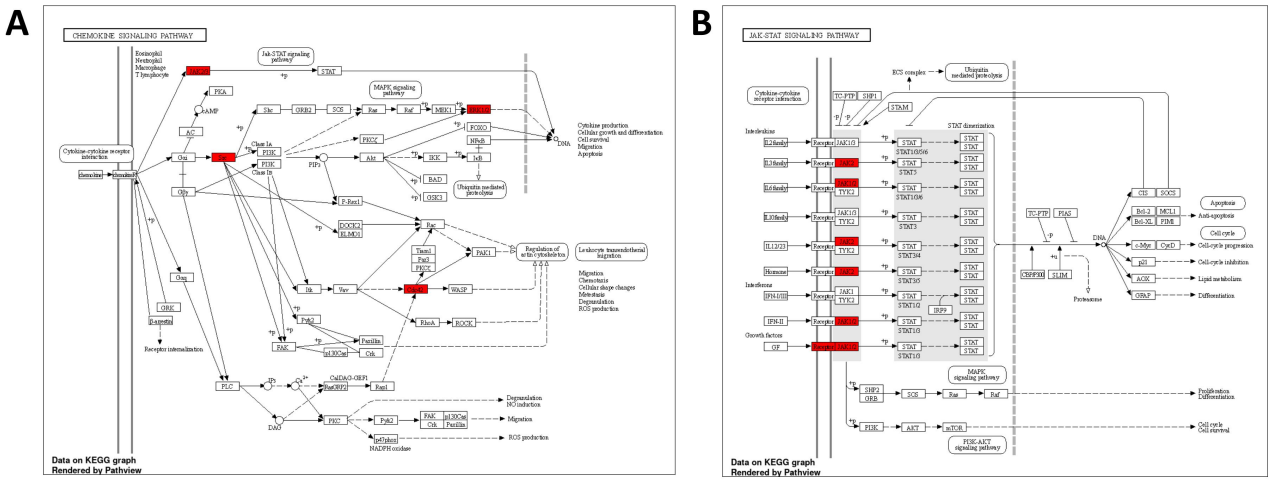

Figure 7 Pathways. (A) Chemokine pathway. (B) Jak-sat pathway.

Molecular docking

The validation of the docking protocol involved eliminating the X-ray-bound ligand from the complex, followed by the redocking process. Subsequently, the RMSD was calculated by comparing the docked conformation of the inhibitor with the X-ray-bound conformation, as illustrated in Figure 8. The validated protocol was subsequently used for further docking. The docking protocol was selected based on RMSD values less than 2 Å for both targets. The selected ligands were successfully docked on the targets to determine their interaction affinity (kcal/mol). Table 7 shows the names of the derivatives, binding affinities (kcal/mol), and interacting residues. Different interactions, such as hydrophobic interactions, hydrogen bond formation and other interactions, include π-stacking and the formation of salt bridges. The binding poses of the ligands with the receptors are shown in Figure 9.

Discussion

In silico-based screening is a highly valuable strategy for the exploration of novel antiviral drugs. The utilization of computational methods for screening natural compounds and phytochemicals through molecular docking studies not only diminishes the resource-intensive aspects of time and finances but also mitigates potential errors that may arise during clinical trials [20]. In contrast to the conventional "one drug, one target" approach in drug design, the emerging field of network pharmacology adopts a multitargeted therapy paradigm. This approach, characterized by the integration of systems biology, network analysis, connectivity, and redundancy, seeks to elucidate the intricate correlations between drugs and diseases. Network pharmacology (NP) studies have proven effective in identifying novel targets and revealing previously unknown signaling pathways interacting with specific compounds. The NP approach not only offers fresh perspectives on the systemic connections among therapeutic targets but also provides a potent and promising tool for comprehending disease mechanisms at the systemic level and discovering potential bioactive ingredients [21].

This study, within this framework, generated a unique network that offers a comprehensive overview of the molecular mechanisms involving natural phytochemicals, thereby contributing to the advancement of our understanding in this field. The resulting network demonstrated the potential of bioactive substances to modulate COVID-19 through intricate interactions involving 121 proteins across multiple pathways. By means of PPI analysis, we identified 60 nodes and 41 edges. Notably, EGFR, SRC, HSP90AA1, HSP90AB1, PTGS2, CDC42, MAPK1, ICAM1, JAK2 and MAPK14 were identified as pivotal genes within the context of COVID-19 regulation. Further investigation through GO analysis and KEGG analysis revealed numerous pathways, in addition to highlighting associations with various other diseases and disorders related to the aforementioned genes. GO enrichment analysis confirmed the direct implication of biological activity in the regulatory mechanisms underlying COVID-19 infection. Furthermore, KEGG pathway analysis revealed the critical roles of the COVID-19 pathway, chemokines, and Jak-sat signaling pathways within the designated network. This observation lends support to the proposition that Berberine and Kuwanon Z may serve as viable candidates for the treatment of COVID-19. In addition to the known COVID-19 pathway, chemokine pathway, and Jak-sat signaling pathway, our investigation revealed the involvement of an array of intricate molecular networks, including but not limited to, endocrine resistance, prostate cancer and Kaposi sarcoma-associated herpes virus infection. These findings collectively suggest a promising, multifaceted approach leveraging BBR and Kuwanon Z as potential multitarget therapeutic agents. Molecular docking investigations revealed that the predicted free energy of binding for the docked ligand varied within the range of −7.3 to −10.5 when the ligand was interacting with JAK2 (PDB ID: 7F7 W). Similarly, for the COVID-19 main protease (PDB: 6LU7), the estimated free energy of binding ranged from −6.7 to −8.1. The binding modes between the compounds and proteins can be explained by hydrophobic interactions, hydrogen bond formation and other interactions, such as π-stacking and the formation of salt bridges. The results indicated that, among the series, Kuwanon Z had the highest affinity for the JAK2 and COVID-19 main protease receptors. Further analyses of the docking score showed that, compared with those of standard drugs, the binding energy of Kuwanon Z was −10.5 kcal/mol greater for JAK2, and the amino acid residues used were LEU551, ILE559, LYS561, LEU579, GLU627, PHE628, VAL629, LYS630, LEU680 and SER698, as determined by comparison with the standard, as depicted in Table 6. Moreover, the binding energy of Kuwanon Z toward the main protease of COVID-19 was −8.1 kcal/mol, and the amino acid residues included LEU551, ILE559, LYS561, LEU579, GLU627, PHE628, VAL629, LYS630, LEU680 and SER698, as shown in Table 6.

Limitations and future work

In this investigation, we present a computational approach aimed at identifying potential compounds for the treatment of SARS-CoV-2, coupled with a predictive mechanism. We recognize several limitations inherent in our current study. Despite diligent efforts to construct extensive drug–target networks from publicly available databases, there is the possibility of incomplete data within the network, and certain drug–target interactions may have biological associations. To increase the precision of our network-based methodologies, it is imperative to comprehensively delineate the virus–host interactome for SARS-CoV-2, specifically focusing on discerning biological effects through the application of functional genomics assays. To bolster the reliability of our findings, conducting

preclinical studies to evaluate in vivo efficacy and potential side effects, validated through cell validation studies, is crucial before advancing to clinical trials. Consequently, it is of paramount importance to subject the predictions derived from network pharmacology to additional experimental validation, thereby substantiating the presented hypothesis in forthcoming research endeavors. This comprehensive approach aims to ensure the robustness and credibility of our methodology.

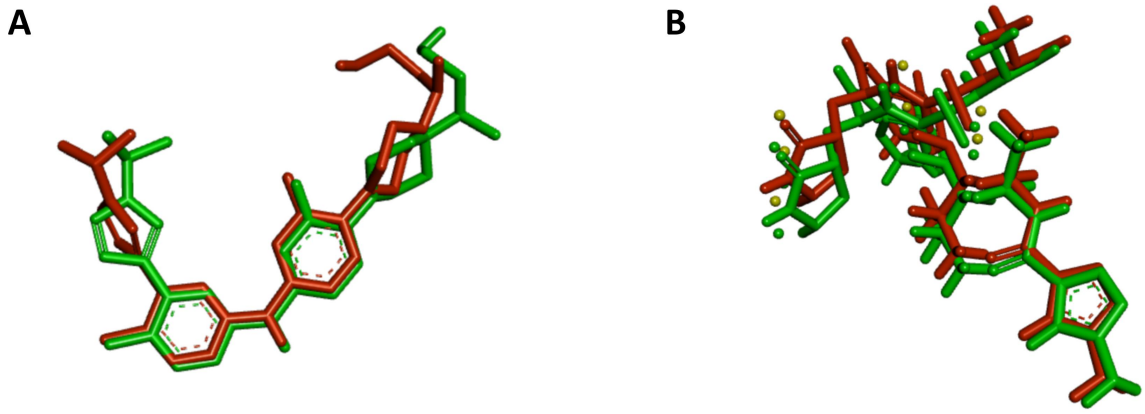

Figure 8 RMSD values. (A) JAK2: RMSD value between X-RAY bound ligand and docked ligand is 1.20 Å. (B) COVID-19 main protease (RMSD: 0.80 Å).

Table 7 Estimated free energy of binding and interacting residues between compounds and receptors

Targets	Compounds	Interacting residues				
		Binding energy	Hydrophobic interactions	H-bonding interactions	π-stacking	Salt bridges
JAK2 (PDB: 7F7W)	Kuwanon	−10.5	LEU551, ILE559 ,LYS561, LEU579, LEU680	LYS561, GLU627, VAL629, LYS630, SER698	PHE628	--
	Berberine	−8.9	LEU551, ILE559, VAL610, GLN626, PHE628, VAL629, LEU680	SER698	--	LYS581
	Remdesivir	−8	LYS561, GLU627, VAL629, LYS630, SER698	LYS581, SER633, THR636, LYS677, ASN678, SER698	--	LYS677
	Nirmatrelvir	−7.5	LEU551, ILE559, PHE628, THR636, LYS640 ,LEU680	VAL629, SER633	--	--
	Molnupiravir	−7.3	LEU551, ILE559	LYS581, GLU627, VAL629, SER633, SER698	--	--
Main protease (PDB: 6LU7)	Kuwanon	−8.1	LEU551, ILE559, LYS561, LEU579, LEU680	LYS561, GLU627, VAL629, LYS630, SER698	PHE628	
	Berberine	−7.7	LEU551, ILE559, VAL610, GLN626, PHE628, VAL629, LEU680	SER698	--	LYS581
	Remdesivir	−7.4	LEU551, LEU579, PHE628, THR636	LYS581, SER633, THR636, LYS677, ASN678, SER698	--	LYS677
	Nirmatrelvir	−7.6	LEU551, ILE559, PHE628, THR636, LYS640, LEU680	VAL629, SER633	--	--
	Molnupiravir	−6.7	LEU551, ILE559	GLN533, LYS581, GLU627, VAL629, SER633, SER698	--	--

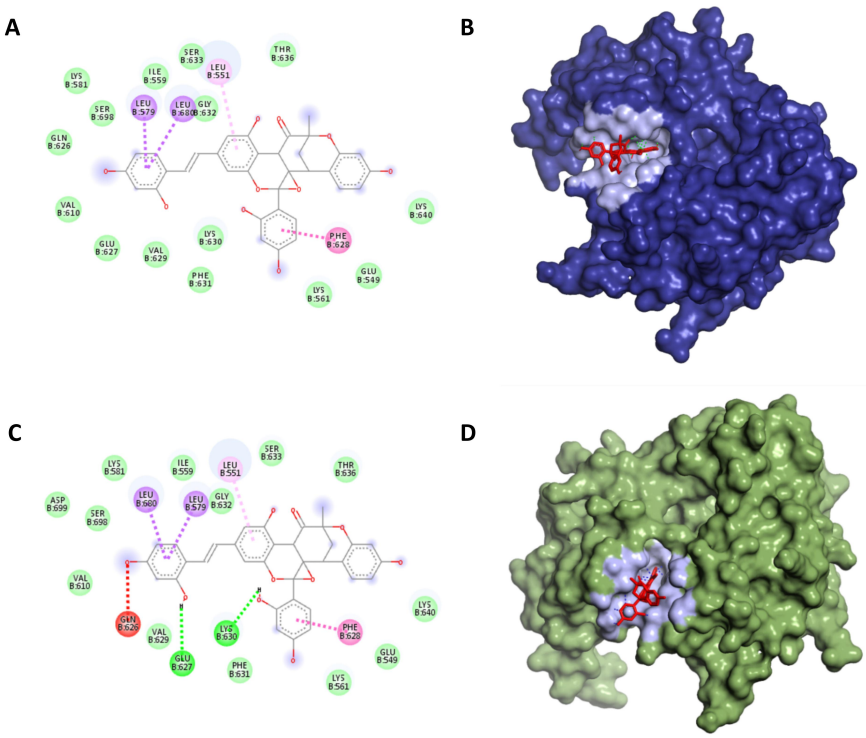

Figure 9 2D and 3D poses of interactions. (A) 2D and (B) 3D poses of interactions between kuwanon Z and target protein JAK2 (PDB ID: 7F7W) and (C) 2D and (D) 3D poses of interactions betweenkuwanon Z and Target protein COVID-19 main protease (PDB: 6LU7).

Conclusion

In the present investigation, we applied a comprehensive approach, encompassing network pharmacology integrated with extensive database mining techniques, to elucidate the principal target proteins associated with Berberine and Kuwanon Z for the amelioration of COVID-19. This involved the establishment of a meticulously constructed network of target proteins. Notably, our findings revealed that the interaction between BBR and Kuwanon Z primarily occurs within the domains of the COVID-19 pathway, chemokines, *Jak-sat*, and other pertinent biological pathways. These actions were shown to modulate crucial target proteins, including EGFR, MET, JAK2, and SRC, thereby exhibiting a significant therapeutic impact on COVID-19. The integration of network analysis studies provides strong support for the unique characteristics of these compounds in COVID-19, indicating their potential as novel pharmacological interventions in COVID-19 management. The findings from our current investigation revealed the noteworthy propensity of Berberine and Kuwanon Z to form robust bonds with COVID-19 targets. Such interactions are indicated to induce alterations in the protein structure, consequently impeding viral function and restricting overall virus activity. Notably, Berberine and Kuwanon Z have emerged as potential inhibitors of this virus protease, positioning them compelling contenders for drug discovery. However, it is imperative to underscore that substantiation of these *in silico* discoveries necessitates further scrutiny through rigorous *in vivo* and in vitro examinations.

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