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Research Article

Pharmacological Mechanism of Zuojin Pill for Gastroesophageal Reflux Disease: A Network Pharmacology Study

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Background. Although Zuojin Pill (ZJP) is widely used in China as a traditional prescription to treat gastroesophageal reflux disease (GERD), its exact mechanism of action is still unknown. Therefore, we employed network pharmacology (NP), molecular docking (MD), and molecular dynamics simulation (MDS) to investigate the pharmacological mechanisms of ZJP against GERD. *Methods*. Active compounds and target genes corresponding to ZJP and target genes related to GERD were identified through analysis of publicly available datasets. Subsequently, the obtained data were subjected to further network pharmacological analysis to explore the potential key active compounds, core target genes, and biological processes (BPs) associated with the effect of ZJP against GERD. Finally, the prediction results of NP were validated by MD, and MDS of the optimal core protein-ligand for each component obtained by MD were performed using Gromacs 2020 software. *Results*. Twelve active components of ZJP were identified to act on 82 target genes associated with GERD, and ZJP might exert an anti-GERD effect through the regulation of BPs such as reactive oxygen species (ROS) metabolism, response to oxidative stress (OS), and ROS, as well as the activation of signaling pathways such as apoptosis, p53 signaling, chemical carcinogenesis-ROS, and HIF-1 signaling pathways. Furthermore, quercetin, kaempferol, and coptisine, the three key components of ZJP were shown to stably bond with the 14 core target genes, including AKT1, MMP2, TP53, EGFR, JUN, CASP3, CXCL8, HIF1α, IL-1β, MYC, PPARG, MMP9, PTGS2, and FOS. Results from MDS showed that PPARG-quercetin and MMP2-quercetin bound more stably. *Conclusions*. The findings suggest that ZJP alleviates the symptoms of GERD and improves the prognosis by regulating ROS metabolism, thereby reducing the secretion of proinflammatory cytokines like IL-1β, COX-2, CXCL8, and MMPs, regulating the expression of oncogenes such as JUN and FOS, and maintaining the normal expression of tumor suppressor genes such as TP53 and MYC. However, whether the effect of this modulation of ROS metabolism is positive or negative needs to be further verified by pharmacological experiments.

1. Introduction

Gastroesophageal reflux disease (GERD) is a digestive disorder with typical symptoms of reflux and heartburn caused by the reflux of gastroduodenal contents into the esophagus [1]. Globally, the prevalence of GERD is estimated to be between 5.2% and 51.2%, with the number of affected individuals on the rise annually [2]. In addition to gastrointestinal discomfort, patients with GERD often exhibit mood changes, such as anxiety and depression, which reduces the quality of life to a great extent [3]. Moreover, GERD is associated with a significant economic burden, with annual healthcare costs for GERD amounting to $1520 billion in the United States [4]. Currently, proton pump inhibitors (PPIs) such as omeprazole and pantoprazole are used as first-line drugs for GERD treatment; however, these drugs are associated with side effects. For example, some studies have pointed out that the use of PPI is correlated with small

intestinal bacterial overgrowth and *Clostridium difficile* infection [5–7]. Therefore, it is imperative to search for better therapeutic strategies against GERD.

Zuojin Pill (ZJP) is made up of Rhizoma coptidis and Evodia rutaecarpa, two functional foods that not only give consumers energy and nourishment but also improve their body's defenses against viral and metabolic disorders. The usage of the famous Chinese drug ZJP was originally documented in the classic text of traditional Chinese medicine (TCM) known as Danxi's Experiential Therapy. In recent years, ZJP has been extensively used in the treatment of GERD in China, and several clinical trials have confirmed its efficacy in improving symptoms, relieving anxiety, and reducing disease recurrence [8, 9]. However, the mechanism of action of ZJP remains unclear.

Network pharmacology (NP) is an emerging scientific method based on the theory of systems biology, integrating pharmacology, and computer technology, which explores the interaction between the nodes of the network and the disease by constructing a "drug-target-disease" network. In the past 15 years, NP has been used by an increasing number of researchers to explore the mechanism of action of herbal components in various diseases [10–13]. Under such inspiration, we analyzed the underlying mechanism by which ZJP works against GERD using a NP approach and validated the results by molecular docking (MD) and molecular dynamics simulation (MDS). The specific workflow is shown in Figure 1.

2. Methods

2.1. Screening for Active Drug Components and Targets. The TCM Systems Pharmacology (TCMSP) [14] was used to retrieve the compounds and targets of R. coptidis and E. officinalis, with oral bioavailability ≥30% and drug-like property ≥0.18 as screening parameters [14]. Gene symbols were normalized for extracted targets using UniProt [15].

2.2. Screening for Disease Targets. The GERD-related targets were retrieved from GeneCards (https://www.genecards.org/), PharmGKB (https://www.pharmgkb.org/), DrugBank (https://www.drugbank.ca/), and TTD (https://db.idrblab.net/ttd/). A correlation score ≥10 was set as the screening criterion for GeneCards targets [16].

2.3. Construction of Drug-Compound-Target Gene Network. By screening and extracting common targets of ZJP and GERD through a Venn diagram, the extracted results were employed as potential targets of action for ZJP in GERD. The drug-compound-target gene network was constructed using Cytoscape 3.7.2 software [17].

2.4. Biofunctional Enrichment Analysis Using Gene Ontology and Kyoto Encyclopedia of Genes and Genomes. To further explore the mechanisms of action of ZJP in GERD, Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of common targets [18] were executed using the ClusterProfiler software package in R 4.0.5.

2.5. Protein-Protein Interaction Analysis and Core Target Identification. Common targets of ZJP and GERD were uploaded to the STRING (https://string-db.org/) website, with confidence >0.4 as the target screening parameter [19]. Then, information on the screened targets was imported into Cytoscape to obtain a protein-protein interaction (PPI) network graph [20]. The degree centrality (DC), closeness centrality (CC), betweenness centrality (BC), network centrality (NC), eigenvector centrality (EC), and local average connectivity (LAC) were calculated to obtain core target genes.

2.6. Molecular Docking. To verify the correlation between compounds and targets, the results of NP were validated by MD in Discovery Studio 2019. The structures of ZJP and macromolecular protein targets associated with GERD receptors were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and PDB (https://www.rcsb.org/) [21, 22]. The LibDock docking conditions were set as follows: docking preference, high quality; conformational method, fast; other parameters, default values. The higher the LibDock score was, the more likely the target binding prediction was.

2.7. Molecular Dynamics Simulation. A simulation of MDS was conducted for the optimal core protein-ligand obtained for each compound of ZZP by molecular docking using Gromacs 2020 software. The conditions were set as follows: force field, Charmm 36; water model, TIP3P; cubic solvent box, side length, 1.2, concomitant type cycle boundary condition, and 1 ns. Subsequently, a 100 ps NVT and 100 ps NPT equilibrium was performed to stabilize the system, and a 100 ns MDS was performed for the whole system. The nonbonded interaction cutoff value was set to 1.2 nm, and the PME algorithm was used to calculate the long-range electrostatic interaction. The time step was set to 2 fs, and one conformation was saved every 10 ps.

3. Results

3.1. Active Components and Targets Associated with ZJP. By searching the compounds and targets of ZJP in the TCMSP database, we obtained a total of 12 active components: 10 for R. coptidis and 2 for E. officinalis. Subsequently, 162 targets related to these 12 active components were retrieved and uploaded to UniProt for gene symbol normalization. The 12 active compounds and 162 targets are detailed in Supplement A.

3.2. Targets Associated with GERD. A total of 4,399 targets associated with GERD were retrieved from the databases Drug-Bank, TTD, PharmGKB, and GeneCards, 118 from Drug-Bank, 12 from TTD, 200 from PharmGKB, and 4,069 from GeneCards. For further analysis, 2,786 targets from GeneCards with scores ≥10 were selected. Subsequently, by merging and eliminating duplicates from the four databases, we finally obtained 1,476 targets. The 1,476 target genes are detailed in Supplement B.

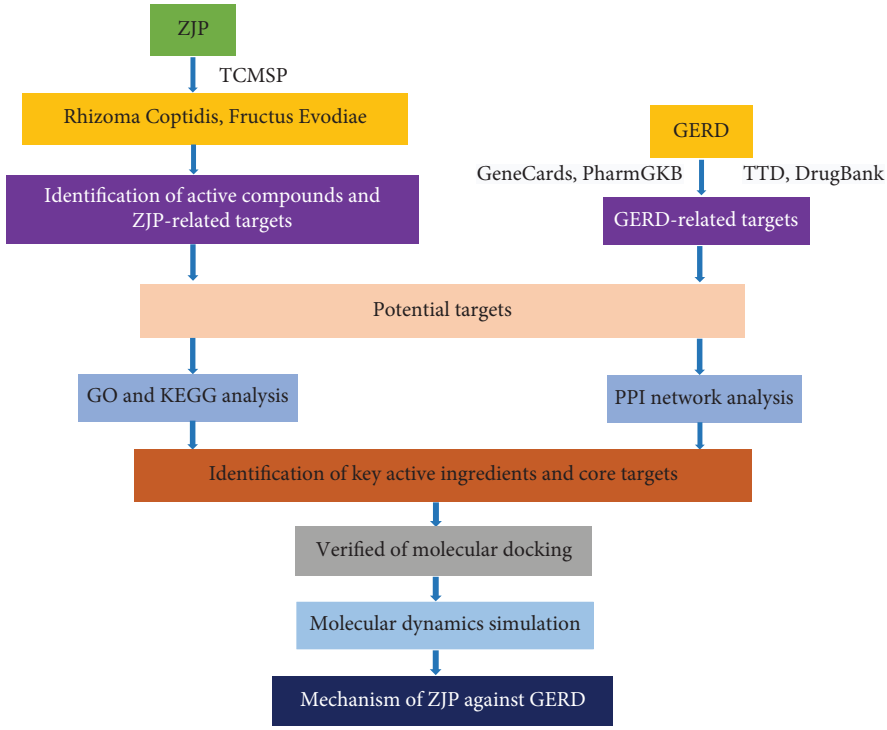

Figure 1: Workflow of the study.

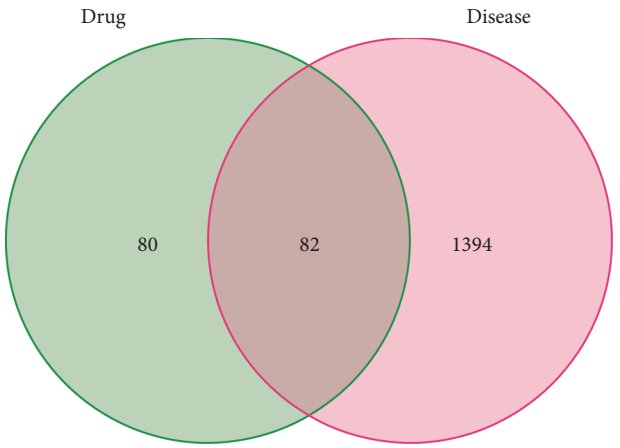

Figure 2: Venn diagram of targets from ZJP and GERD.

3.3. Construction of Drug-Compound-Target Gene Network. As shown in the Venn diagram (Figure 2), 82 common target genes of GERD and ZJP were identified. Based on these 82 common target genes, a drug-component-target gene network consisting of 12 components, 82 target genes, 97 nodes, and 165 edges was constructed. Details of this network are shown in Figure 3.

3.4. GO and KEGG Bifunctional Enrichment Analyses. Results of GO analysis (Figure 4) revealed that common targets of ZJP and GERD were mainly enriched in biological processes (BPs) such as cellular response to chemical stress, oxidative stress (OS), reactive oxygen species (ROS), and reactive ROS metabolic process and. Furthermore, KEGG analysis showed that ZJP mainly affected GERD by inhibiting apoptosis, Th17 cell differentiation, chemical carcinogenesis-ROS, p53, HIF-1, TNF, and IL-17 signaling pathways.

3.5. PPI Network and Core Gene Analyses. The PPI network, consisting of 81 nodes and 2498 interactions, was constructed using the STRING website, as shown in Figure 5(a). In Cytoscape Plugin cytoHubba, the PPI network was subjected to two rounds of screening, and BC, CC, DC, EC, NC, and LAC were calculated (Figures 5(b) and 5(c)). The PPI network in Figure 5(b) contains 31 nodes and 418 interactions, while that in Figure 5(c) contains 14 nodes and 182 interactions. The following target genes corresponding to these 14 nodes were identified: AKT1, MMP2, TP53, EGFR, JUN, CASP3, CXCL8, HIF1A, IL-1β, MYC, PPARG, MMP9, PTGS2, and FOS.

3.6. Molecular Docking. MD was performed to validate NP's results, which are shown in Table 1. The LibDock docking scores of all key active ingredients with corresponding core targets were greater than 80 points (two- and three-dimensional models of them are shown in Figures 6 and 7, respectively), thus indicating a good docking score.

3.7. Molecular Dynamics Simulation. According to the results of MD, MDS was performed for PPARG-quercetin, MMP2-quercetin, AKT1-kaempferol, and PTGS2-coptisine. Results of root mean square deviation, root mean square fluctuation, radius of gyration, and Hbond from MDS showed that PPARG-quercetin, and MMP2-quercetin

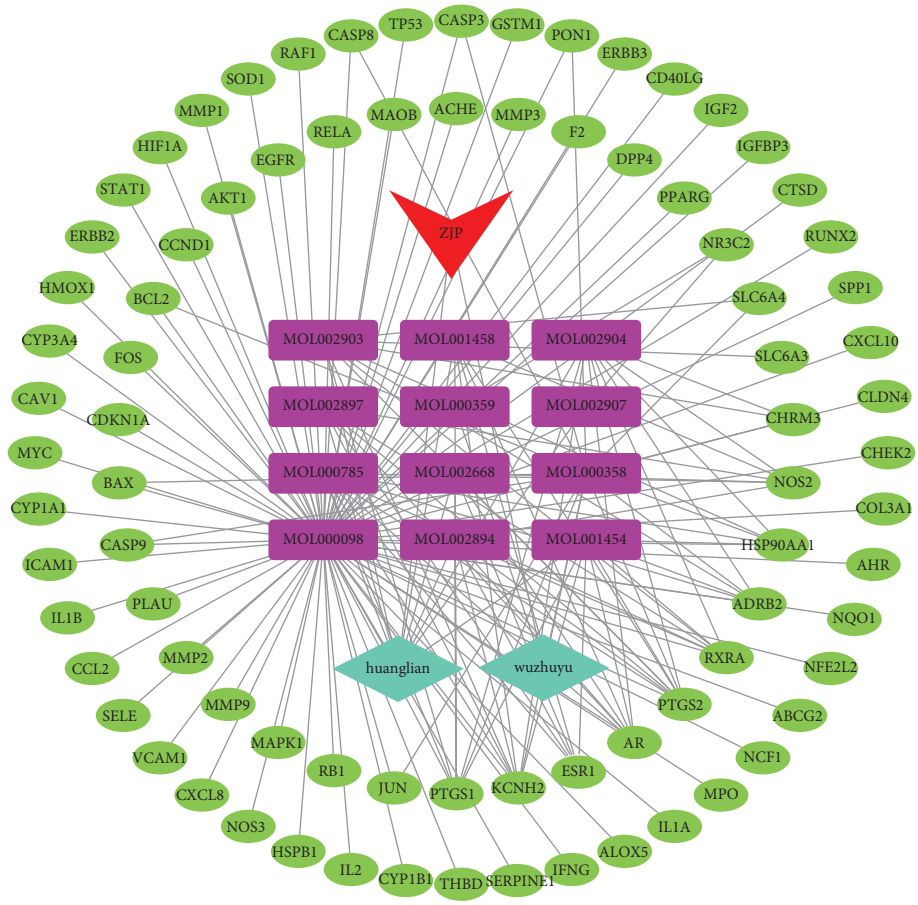

Figure 3: Drug-compound-target gene network of ZJP.

bound more stably, while AKT1-kaempferol, and PTGS2-coptisine did not form stable complex. Details are presented in Figure 8.

4. Discussion

The increasing prevalence of GERD, combined with a greater understanding of the side effects of PPI drugs [2, 23, 24], has led to an active search for better treatment modalities against GERD. As a traditional Chinese prescription drug, ZJP has been widely used in the treatment of GERD with a remarkable efficacy. However, due to the complexity of herbal compounds, the mechanism of action of ZJP in the treatment of GERDs is still unclear. Therefore, in this study, we applied NP, MD, and MDS to analyze the mechanism of action of ZJP in GERD.

The drug-target-disease network of ZJP and GERD included 12 compounds, 82 targets, and 165 pathways of action. Results of GO analysis revealed that these genes were associated with BPs such as cellular response to chemical stress, response to OS, ROS metabolic process, and other aspects related to OS reaction and ROS metabolism. In recent years, ROS has also been found to regulate apoptosis, expression of tumor suppressor genes (such as p53), induction of HIF-1 activation, and synthesis of proinflammatory factors (such as IL-1β) [25–28], which overlapped with the results of KEGG analysis. To dig deeper into these mechanisms, we constructed a PPI network and screened out 14 core targets, namely, AKT1, MMP2, TP53, EGFR, JUN, CASP3, CXCL8, HIF1A, IL-1β, MYC, PPARG, MMP9, PTGS2, and FOS. Subsequently, the good docking relationship between quercetin, kaempferol, coptisine, the key active components of ZJP and the 14 core targets was performed by MD. In the 60 ns MDS, quercetin formed the most stable complex with the MMP2 and the second most stable with the PPARG.

The pathogenesis of GERD is relatively complex. GERD is characterized by transient relaxation of the subesophageal sphincter, elevated abdominal pressure, decline in the esophageal clearance ability, and high sensitivity of the esophagus. Moreover, the mechanisms of GERD pathogenesis include the release of gastric and duodenal contents such as gastric acid, protease, bile salts, and other chemicals, which can cause esophageal mucosa stimulation [29]. Gastric acid reflux is widely recognized as a symptom of GERD. However, the severity of GERD symptoms and the amount of acid reflux do not strictly correlate with one another, as evidenced by improvements in clinical trials and basic experiments [30]. Moreover, the pathological changes related to nonerosive reflux disease may occur in the proximal esophageal mucosa without contact with gastric contents. Additionally, 10–40% of patients with GERD do not exhibit the desired outcome after PPI treatment [30, 31], suggesting that the pathogenesis of GERD still needs to be explored. Studies over the past 20 years have demonstrated

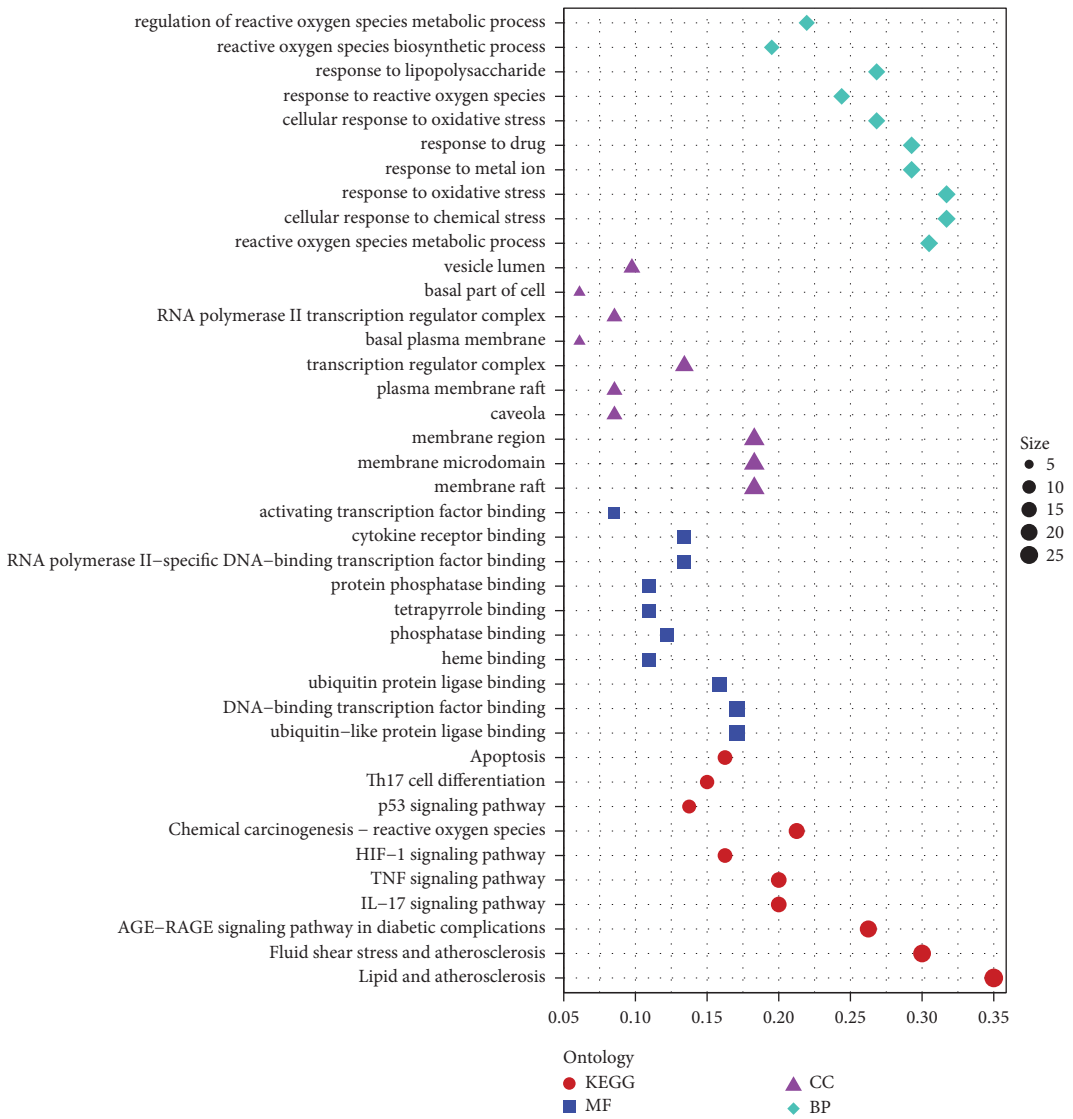

Figure 4: Results of GO and KEGG biofunctional enrichment analysis.

that reflux can induce an esophageal inflammatory reaction, causing the inflammatory cells in the esophageal epithelium to release inflammatory factors and trigger OS response, thus leading to a loss of the dynamic balance between oxidant and antioxidant activities. Therefore, ROS accumulate in large quantities in the esophageal mucosa, damaging the membrane structure and causing esophageal mucosal injury [32–34]. Therefore, these results indicate that OS plays a nonnegligible role in GERD pathogenesis. Thus, inhibition of OS or reduction of tissue damage by OS may be a key therapeutic strategy against GERD. In GO analysis, the terms OS and ROS appeared more frequently, which was in agreement with the results of previous studies. Meanwhile, many previous studies have confirmed that ROS plays an important role in regulating apoptosis, tumor suppressor gene expression, induction of HIF-1 activation, and synthesis of proinflammatory factors (such as IL-1β) [25–28], which were consistent with the results of KEGG analysis. Therefore, ZJP may play a therapeutic role in GERD by regulating ROS metabolism and OS response.

Quercetin, kaempferol, and coptisine were analyzed as key active components of ZJP action in GERD. Previous reports have indicated that quercetin reverses the expression of antioxidant enzymes during the OS response and induces the expression of heme oxygenase-1 [35]. Meanwhile, several derivatives of quercetin such as quercetin-3-O-glucoside can regulate the active site of oxidative damage *in vivo* and protect primitive cells from acute OS [36]. Kaempferol, a natural flavonol, could reduce the lipase-induced overproduction of TNF-α, IL-1β, and IL-6 by reducing intestinal inflammation, thereby playing a negative regulatory role in the progression of intestinal inflammation [37]. In addition, kaempferol could reduce cellular damage caused by OS through its antioxidant activity and antiapoptotic function [38], and this antioxidant effect was enhanced by its reactivity with metal ions [39]. Coptisine, a major component of R. coptidis, can inhibit the AMP-activated protein kinase (AMPK) pathway and mitogen-activated protein kinase (MAPK) by inhibiting AMPK in macrophages and the MAPK signaling pathway. Moreover, coptisine can reduce

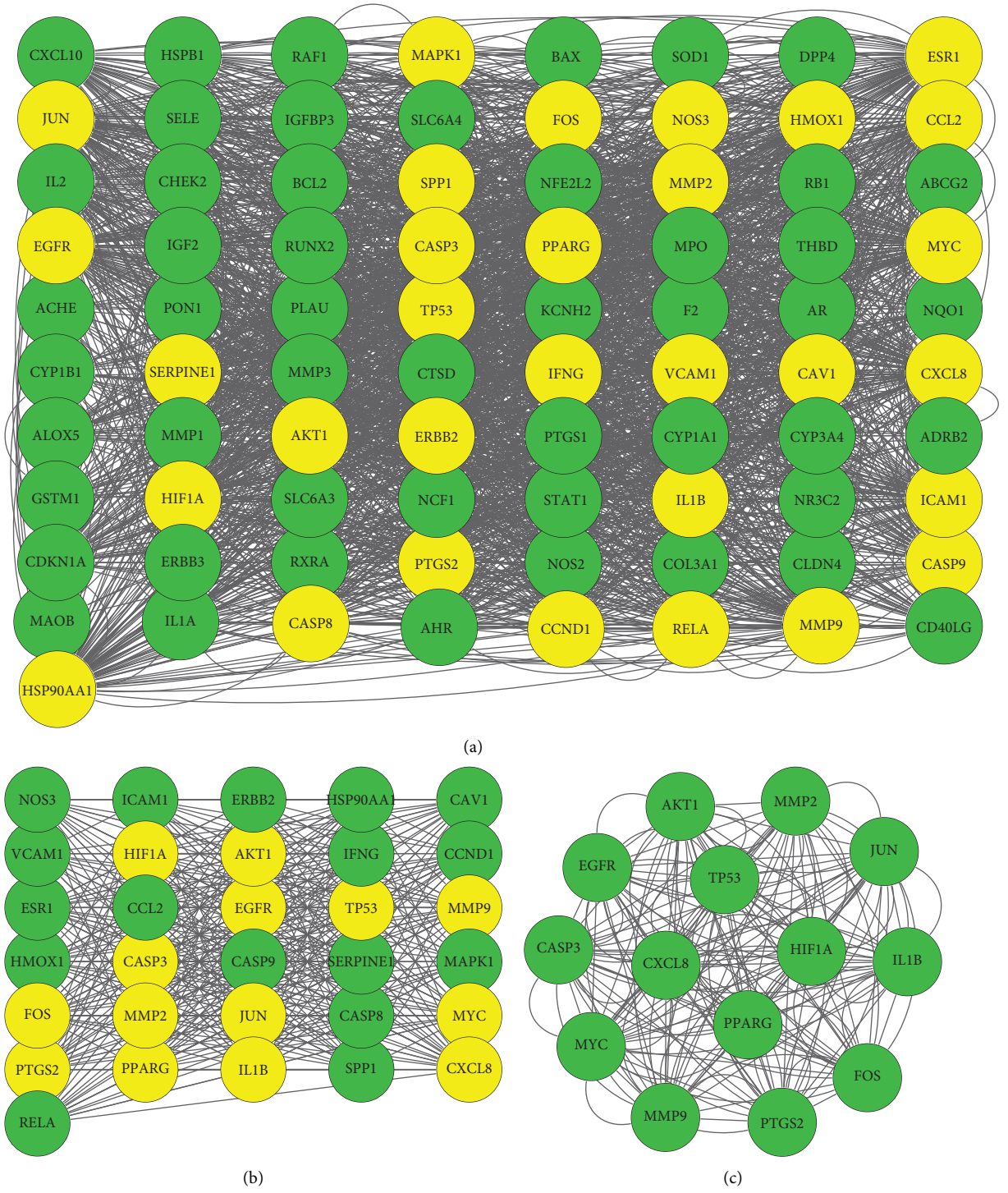

Figure 5: Process of topological screening for the PPI network. (a) PPI network from STRING visualized with Cytoscape. (b) PPI network of more significant proteins extracted from (a) by filtering 6 parameters: BC, CC, DC, EC, NC, and LAC. (c) Core PPI network of core targets extracted from (b).

Table 1: Results of molecular docking.

Query	Core genes	PDB ID	Ingredients	LibDock score
1	IL1B	5MVZ	Quercetin	105.093
2	EGFR	2EB2	Quercetin	93.095
3	TP53	6WQX	Quercetin	113.076
4	MYC	1EE4	Quercetin	105.596
5	JUN	1A02	Quercetin	85.964
6	HIF1A	4H6J	Quercetin	95.596
7	CASP3	1CP3	Quercetin	84.239
8	CXCL8	1QE6	Quercetin	115.326
9	PPARG	1K74	Quercetin	126.302
10	MMP9	1GKC	Quercetin	112.681
11	MMP2	1QIB	Quercetin	130.113
12	FOS	1FOS	Quercetin	98.384
13	AKT1	3MV5	Kaempferol	105.962
14	PTGS2	5ikq	Coptisine	101.335

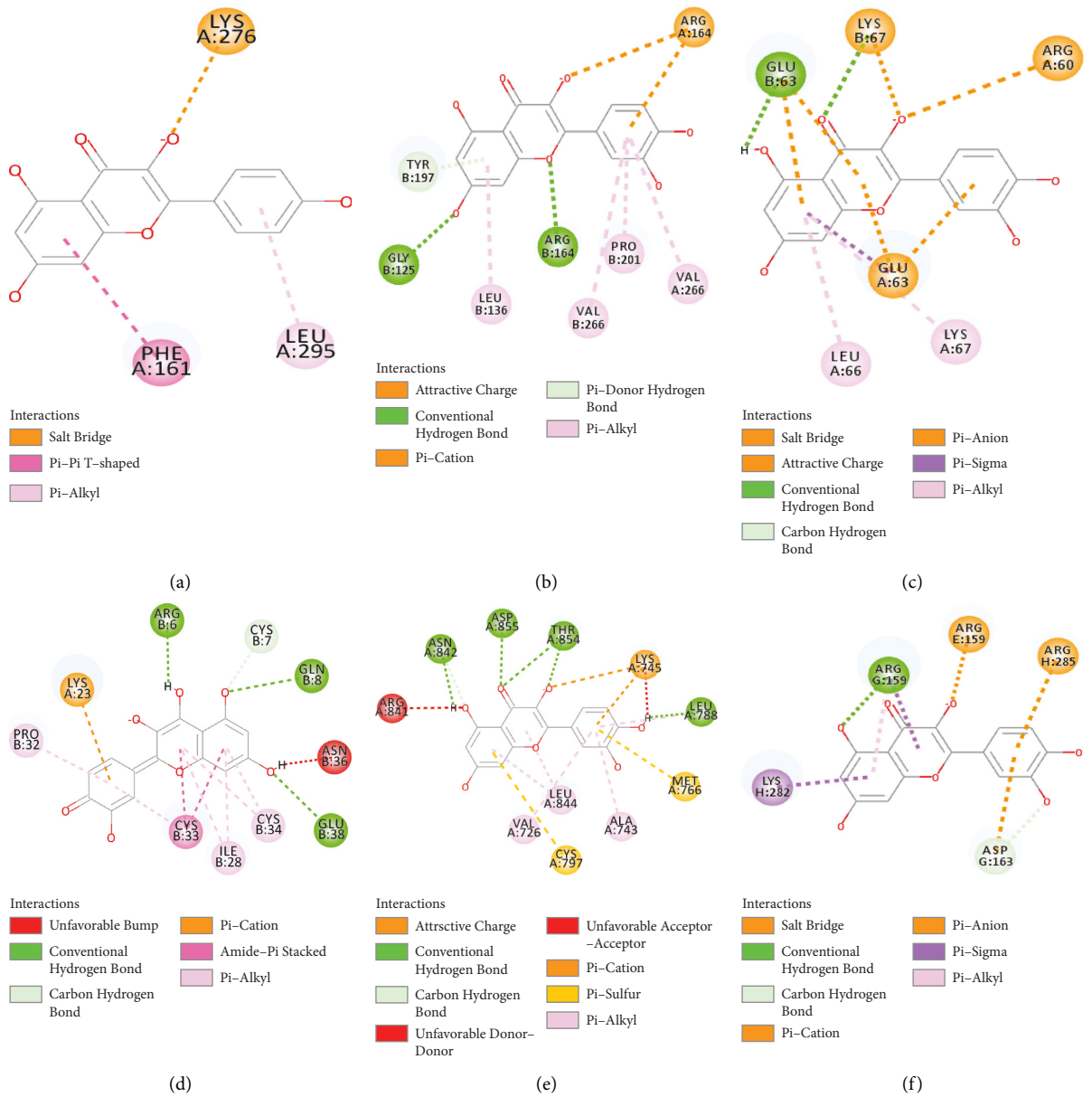

Figure 6: Continued.

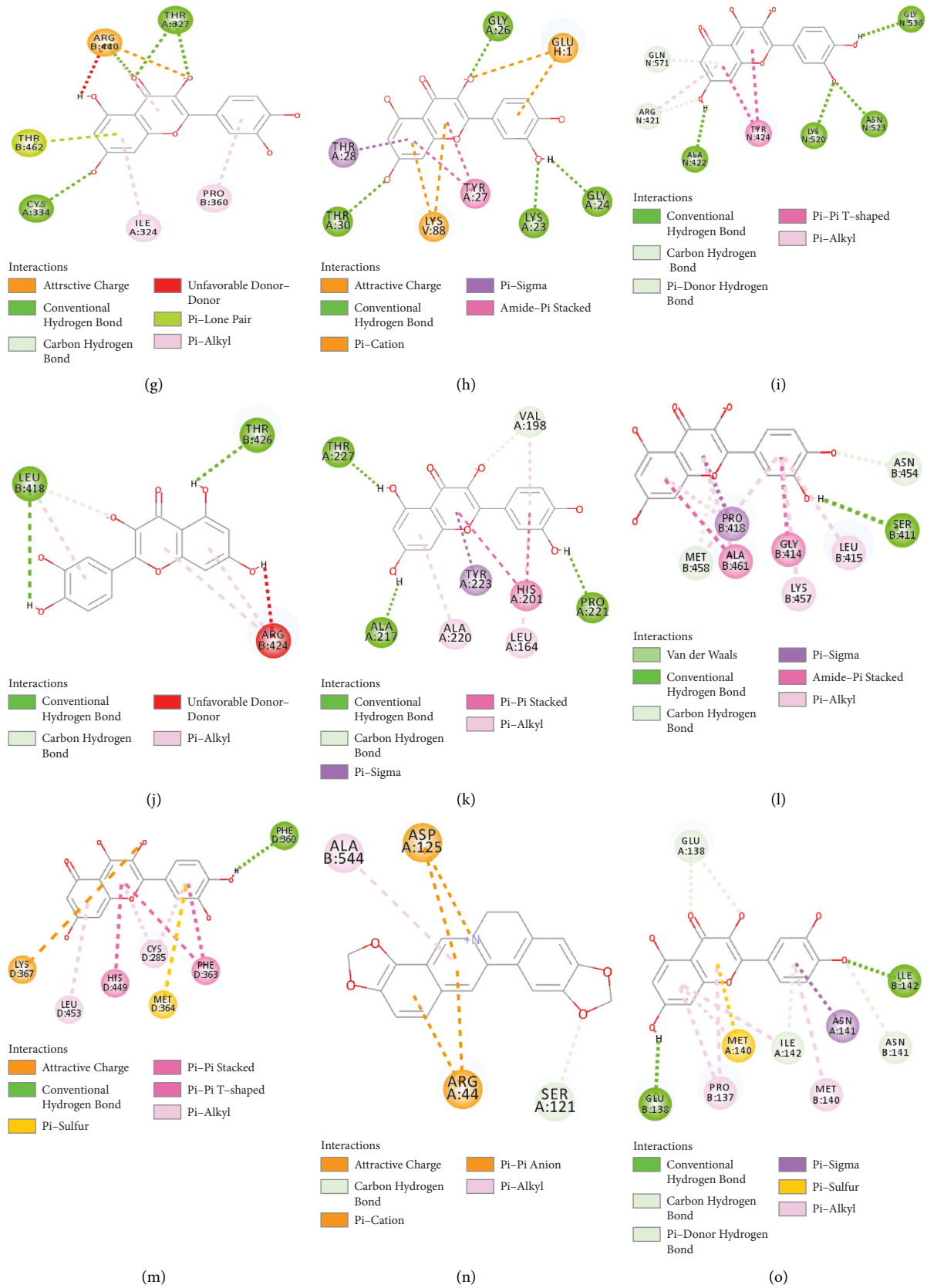

Figure 6: Two-dimensional molecular docking model. (a) AKT1-3MV5; (b) CASP3-1CP3; (c) CXCL8-1ICW; (d) CXCL8-1QE6; (e) EGFR-2EB2; (f) FOS-1FOS; (g) HIF1A-4H6J; (h) IL1B-5MVZ; (i) JUN-1A02; (j) MMP9-1GKC; (k) MMP21QIB; (l) MYC-1EE4; (m) PPARG1K74; (n) PTGS2-5IKQ; and (o) TP536WQX-1.

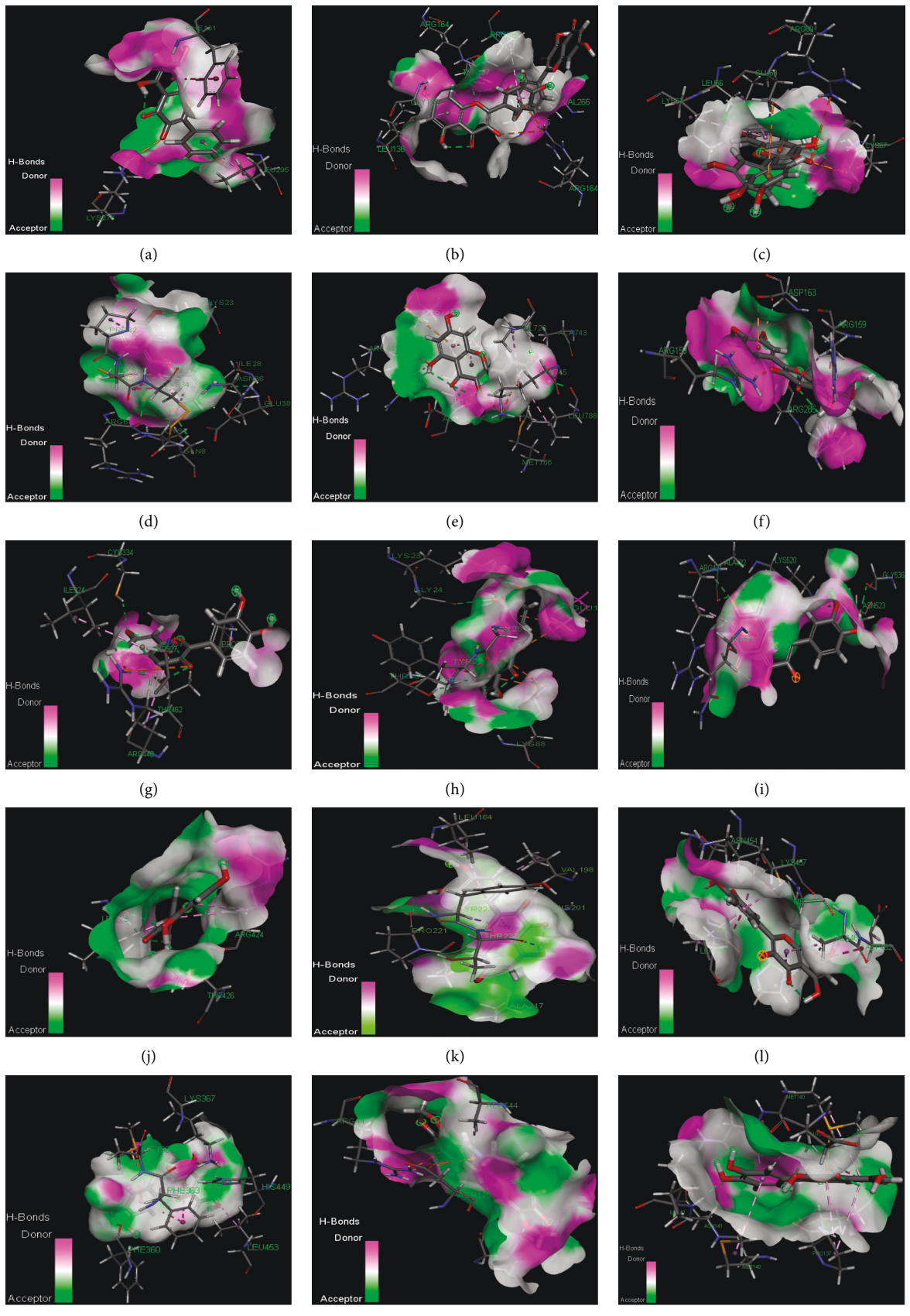

Figure 7: Three-dimensional molecular docking model. (a) AKT1-3MV5; (b) CASP3-1CP3; (c) CXCL8-1ICW; (d) CXCL8-1QE6; (e) EGFR-2EB2; (f) FOS-1FOS; (g) HIF1A-4H6J; (h) IL1B-5MVZ; (i) JUN-1A02; (j) MMP9-1GKC; (k) MMP21QIB; (l) MYC-1EE4; (m) PPARG1K74; (n) PTGS2-5IKQ; and (o) TP536WQX-1.

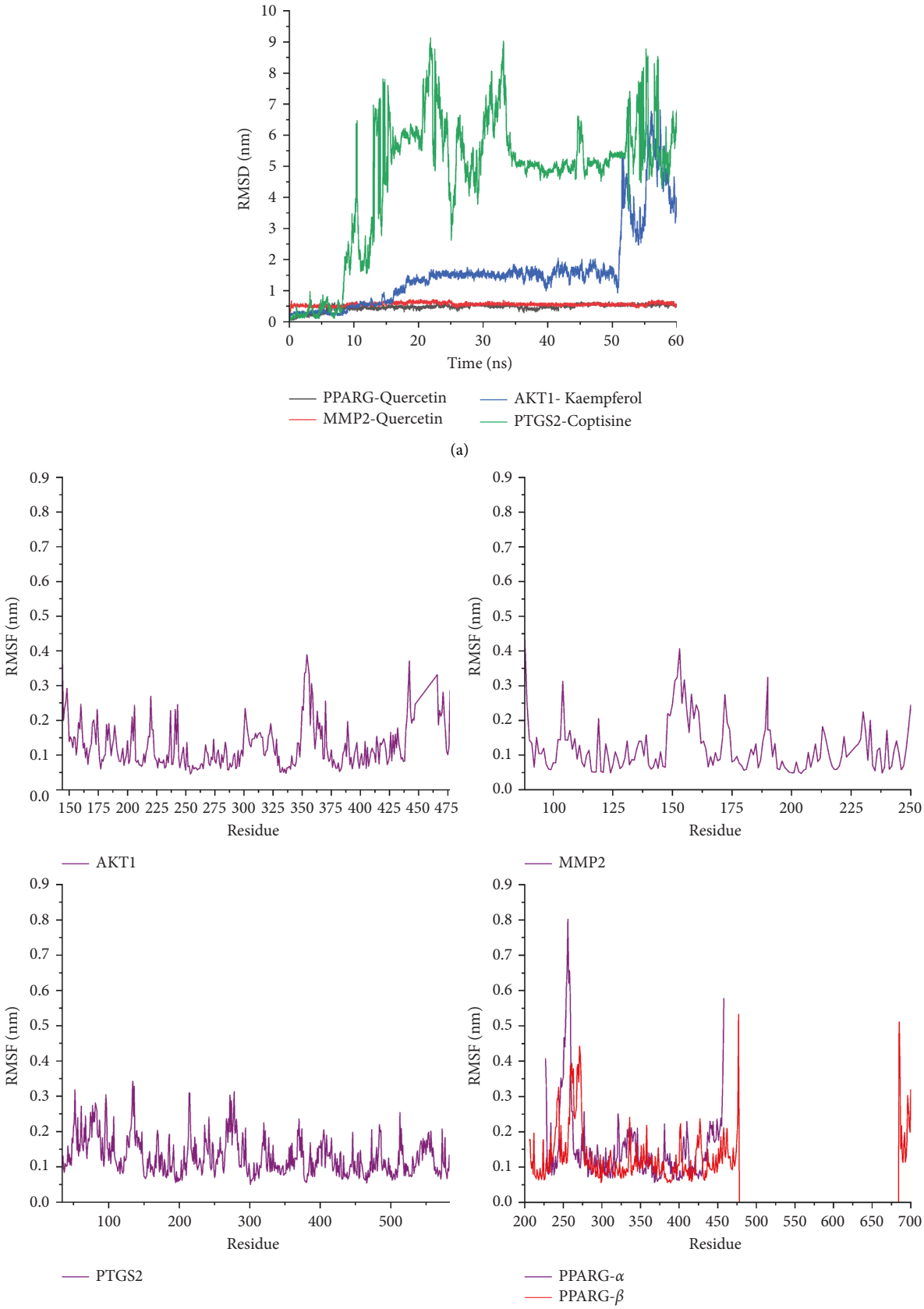

Figure 8: Continued.

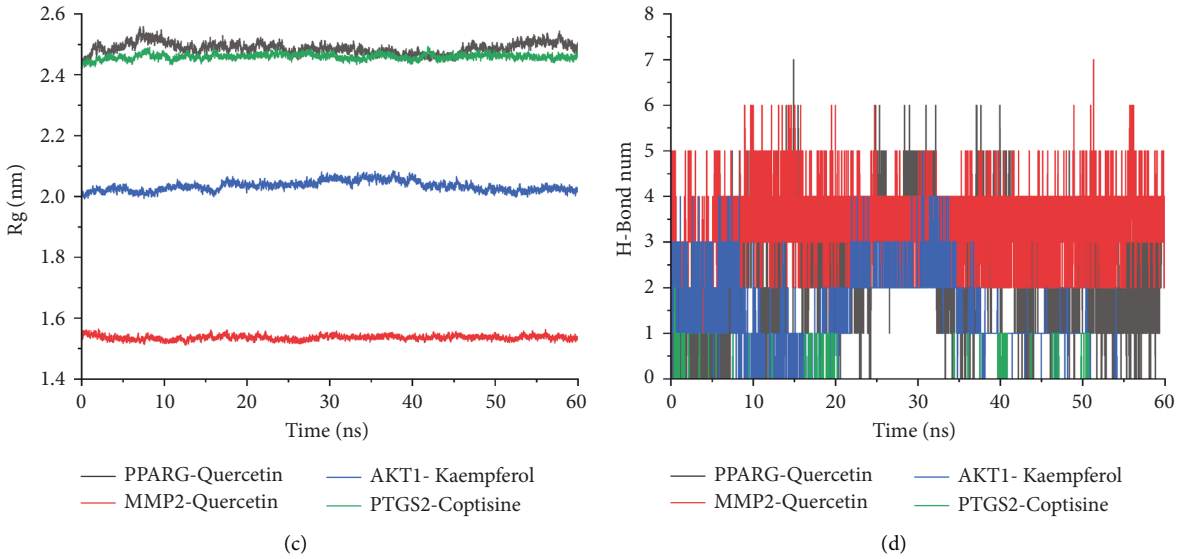

Figure 8: Results of molecular dynamics simulations. (a) Root mean square deviation (RMSD); (b) root mean square fluctuation (RMSF); (c) radius of gyration (Rg); (d) Hbond.

the inflammatory response by downregulating the expression of inflammatory factors such as TNF-α, IL-1β, MCP-1, and MMP-9 [40–42] and inhibit the inflammatory and OS responses by activating the Nrf2 pathway [43]. These results provide further evidence that ZJP is an effective therapeutic agent against GERD.

This study has clearly highlighted some of the multiple components found within ZZP and found some of its targets which may be involved in its beneficial effects upon GERD. However, limited by the deficiencies of systems biology, multidirectional pharmacology, computational biology, and network analysis, this study provided only preliminary predictions, further validation by clinical and basic experiments is still necessary.

5. Conclusions

This study revealed the possible targets and pathways of ZJP in the treatment of GERD, reflecting the characteristics of multicomponent, multitarget, and multipathway mode of action of ZJP, a Chinese herbal compound, and provided new ideas for further discussion.

Abbreviations

ZJP: Zuojin Pill
GERD: Gastroesophageal reflux disease
NP: Network pharmacology
MD: Molecular docking
MDS: Molecular dynamics simulation
BPs: Biological processes
ROS: Reactive oxygen species
OS: Oxidative stress
PPIs: Proton pump inhibitors
TCM: Traditional Chinese medicine
TCMSP: TCM Systems Pharmacology
GO: Gene ontology
KEGG: Kyoto encyclopedia of genes and genomes
PPI: Protein-protein interaction
DC: Degree centrality
CC: Closeness centrality
BC: Betweenness centrality
NC: Network centrality
EC: Eigenvector centrality
LAC: Local average connectivity
OS: Oxidative stress
ROS: Reactive oxygen species
AMPK: AMP-activated protein kinase
MAPK: Mitogen-activated protein kinase.

Data Availability

All data obtained or analyzed during this work are included within the article.

Disclosure

Mi Lv and Jinke Huang are the co-first authors.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Authors' Contributions

Mi Lv and Jinke Huang initiated the study design and drafted the manuscript. Jiayan Hu, Wenxi Yu, Ping Liu, and Kunli Zhang helped with the implementation of this work. Fengyun Wang contributed to supervision, review, and editing and Xudong Tang contributed to methodology, review, and editing. All authors read and approved the final manuscript.

Acknowledgments

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Supplementary Materials

(1) Active components and targets associated with ZJP.by searching the compounds and targets of ZJP in the TCMSP database shown in Supplementary A. (2) Targets associated with GERD shown in Supplementary B. (*Supplementary Materials*)

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