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DATABASE ANALYSIS

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# A Network Pharmacology Analysis of the Active Components of the Traditional Chinese Medicine Zuojinwan in Patients with Gastric Cancer

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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**Background:** Zuojinwan (ZJW) is a traditional Chinese prescription normally used for gastritis. Several studies indicated that it could fight against gastric cancer. This study was designed to determine the potential pharmacological mechanism of ZJW in the treatment of gastric cancer.

**Material/Methods:** Bioactive compounds and potential targets of ZJW and related genes of gastric cancer were retrieved from public databases. Pharmacological mechanisms including crucial ingredients, potential targets, and signaling pathways were determined using protein-protein interaction (PPI) and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. Virtual docking was performed to validate the findings.

**Results:** Network analysis identified 47 active ZJW compounds, and 48 potential ZJW target genes linked to gastric cancer. Quercetin, beta-sitosterol, isorhamnetin, wogonin, and baicalein were identified as potential candidate agents. Our PPI analysis results combined with previously published results indicated that matrix metalloproteinases family members MMP9, MMP1, and MMP3 may play key roles in the anti-gastric cancer effect of ZJW. Molecular docking analysis showed that these crucial targets had good affinity for the representative components in ZJW. GO and KEGG enrichment analysis showed that ZJW target genes functioned in multiple pathways for treating gastric cancer, including interleukin-17 signaling and platinum drug resistance.

**Conclusions:** Our results illuminate the active ingredients, associated targets, biological processes, and signaling pathways of ZJW in the treatment of gastric cancer. This study enhances our understanding of the potential effects of ZJW in gastric cancer and demonstrates a feasible method for discovering potential drugs from Chinese medicinal formulas.

**MeSH Keywords:** **Medicine, Chinese Traditional • Pharmacology • Stomach Diseases**

**Abbreviation:** **ZJW** – Zuojinwan; **TCM** – Traditional Chinese Medicine; **TCMSP** – Traditional Chinese Medicine Systems Pharmacology; **BATMAN-TCM** – Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine; **OB** – oral bioavailability; **DL** – drug-likeness; **GO** – gene ontology; **KEGG** –Kyoto Encyclopedia of Genes and Genomes; **PPI** – protein–protein interaction; **BP** – biological process; **CC** – cell component; **MF** – molecular function

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## Background

Gastric cancer is the fifth most common malignancy and the third leading cause of cancer-related death worldwide [1]. Yearly, more than 950 000 patients are diagnosed with gastric cancer [2]. More than half of the world's stomach cancer occurs in East Asia, and the overwhelming majority occurs in China. To date, no globally acceptable standard regimen for the treatment of advanced gastric cancer has been established [3]. Neoadjuvant chemotherapy, as well as various chemotherapeutic agents, have shown efficacy in the treatment of gastric cancer [4,5]. However, there are 2 unavoidable barriers to successful treatment: tumor cell resistance to anticancer agents and systemic damage of chemotherapeutic agents [6]. Hence, it remains necessary to explore novel, safe, and effective strategies for the treatment of gastric cancer.

Zuojinwan (ZJW) is a traditional Chinese prescription formed by Rhizoma coptidis (R. coptidis, Chinese name: Huanglian) and Euodia rutaecarpa (E. rutaecarpa, Chinese name: Wuzhuyu) at a ratio of 6: 1. ZJW was first mentioned in a famous ancient medicine treatise, "*Dan-Xi-Xin-Fa*", of the Yuan dynasty. R. coptidis is the dried rhizome of Coptis chinensis Franch. and is widely used in the treatment of gastrointestinal disorders, hepatic damage, and diabetes [7]. E. rutaecarpa is obtained from the immature fruit of Evodia rutaecarpa Benth. and is widely used in the treatment of various diseases, including headache, inflammation, and hypertension [8]. Accumulating evidence suggests that ZJW, its comprising herbs R. coptidis and E. rutaecarpa, and its active compounds possess a certain anti-tumor effects [9,10]. ZJW is used as a synergistic drug for patients with cisplatin (DDP)-resistant gastric cancer [11]. Furthermore, ZJW can be used as an inhibitor of chemoresistance in gastric cancer, which may partly be due to dephosphorylation of p-cofilin-1 [12], regulation of ROCK/PTEN/PI3K signaling [13], and induction of mitochondrial apoptosis and AKT inactivation [14]. Despite these previous studies, the active compounds within ZJW, their anti-gastric cancer effects, potential targets, and molecular mechanism remain largely unknown.

Traditional Chinese medicine (TCM) theory is characterized by holistic and systematic concepts. In TCM, it is widely believed that many components act on a variety of cellular targets in multiple pathways for therapeutic purposes. The emergence of network pharmacology, with characteristics consistent with the holistic view of TCM, makes it possible to elucidate the complex network of relationships between TCM and diseases [15–18]. Network pharmacology can reveal the interactions between targets of compounds in herbs and genes associated with specific diseases [19–21].

In this study, a network pharmacology-based approach, combined with molecular docking analysis, was used to determine the active ingredients, potential targets, and pharmacological mechanism of ZJW in the treatment of gastric cancer (Figure 1). Firstly, we searched for information of bioactive compounds in ZJW, and identified candidate target genes for these compounds using public databases. Then, the target genes of gastric cancer were obtained from multiple databases. Subsequently, the ZJW target genes and related signaling pathways against gastric cancer were revealed. The composite compounds of ZJW and the corresponding major target candidates were further validated by molecular docking analysis.

## Material and Methods

### Composite compounds of ZJW

The information about compounds from "R. coptidis" and "E. rutaecarpa" in ZJW were identified through searching the following chemical databases: Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (*http://lsp.nwu.edu.cn/tcmsp.php*) [22]; Traditional Chinese Medicine Database@Taiwan (*http://tcm.cmu.edu.tw/zh-tw/*) [23]; and Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine (BATMAN-TCM) (*http://bionet.ncpsb.org/batman-tcm/*) [24]. In brief, the online databases are searched using the name of the herbal medicine to obtain corresponding compound information, and then all ingredients of the herbal medicine were determined by removing the duplicate ingredients.

### Pharmacokinetic prediction

Most TCM ingredients cannot reach the specific protein targets in cells due to their lack of suitable pharmacological properties. Oral bioavailability (OB) is one of the most important pharmacokinetic parameters in the ADME (absorption, distribution, metabolism, and excretion) properties of drugs. OB is indispensable for determining whether a compound in a Chinese medicine prescription has pharmacological activity [25]. Accurate early evaluation of the drug-likeness (DL) properties of compounds can help to identify if a compound is chemically suitable for use as a drug. Evaluation of the DL of a molecule is related to parameters affecting its pharmacodynamics and pharmacokinetic profiles, which ultimately impact its ADME properties [26]. In the present study, the specific OB and DL values were collected from the TCMSP database. Compounds with OB ≥30% and DL ≥0.18 were selected for further analysis.

### Prediction of drug targets for ZJW

The putative targets of active components in ZJW were obtained from the TCMSP, a database which also includes detailed drug targets and their relationships to disease [22]. We limited the species of target proteins to "Homo sapiens" and obtained their corresponding official symbols through UniProtKB.





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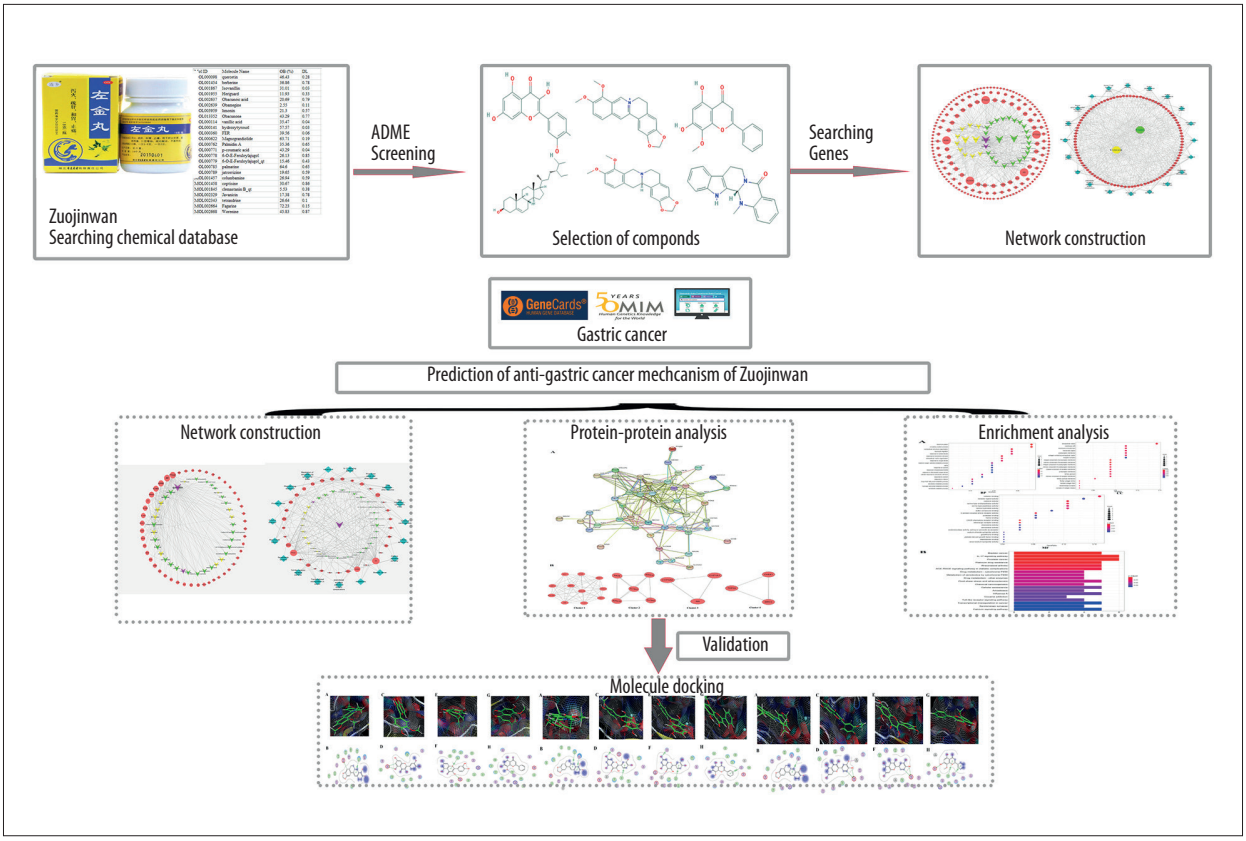


**Figure 1.** Schematic of the network pharmacological analysis used to identify the potential mechanism of Zuojinwan action on gastric cancer.

### Collection of gene targets for gastric cancer

Disease targets were retrieved from GeneCards (*http://www.genecards.org*) and Online Mendelian Inheritance in Man (OMIM, *http://www.omim.org*) [27]. We searched these databases using the keywords "gastric cancer" and "stomach cancer". Then, the putative target genes of ZJW were mapped to the gastric cancer identified targets. Lastly, the candidate anti-gastric cancer targets of ZJW were identified.

### Validation of candidate targets in The Cancer Genome Atlas (TCGA) database

Stomach cancer genes from The Cancer Genome Atlas (TCGA) database were used to validate candidate targets of ZJW against gastric cancer. Firstly, gene expression quantification data form gastric cancer samples were downloaded from the TCGA database (retrospect to April 1, 2019). Then, "edgeR", a Bioconductor package based on R language (Version 3.26.0, *http://www.r-project.org*), was applied to identify the differentially expressed mRNAs (DEmRNA) between tumor tissues and adjacent non-tumor gastric tissues. DEmRNAs with absolute values of log2 Fold Change >1.5 and adjusted *P*-values <0.05 were identified for subsequent validation. Subsequently, a Venn diagram was used to intersect the candidate targets of ZJW against gastric cancer with the gastric cancer DEmRNA obtained from the TCGA database. The intersecting genes were considered potential targets of ZJW against gastric cancer.

### Enrichment analysis

To explore the gene functions, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted using "cluster profile" [28], a Bioconductor package based on R language. The Benjamini-Hochberg method was used to adjust the *P* value [29]. Only functional annotations with an enrichment *P*-value less than 0.05 were chosen for further analysis.

### Protein–protein interaction (PPI)

Core regulatory genes can be identified by studying the protein-protein interaction (PPI). There are many databases related to PPI, and the STRING database (*https://string-db.org/*) covers the greatest number of species and has more interactive information [21]. In this study, the obtained target was submitted to STRING, with the species limited to "Homo sapiens" and confidence scores limited to those >0.4, and the PPI data were extracted.





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### Network construction

Four networks were constructed: 1) active compound-target network of ZJW; 2) potential compounds-potential targets network of ZJW against gastric cancer; 3) potential compounds-potential targets-pathways network of ZJW against gastric cancer; and 4) PPI network of the potential targets of ZJW against gastric cancer. The PPI network was completed directly on STRING (version: 11.0, *www.string-db.org*), and the other 3 networks were constructed using the Cytoscape network visualization software (*http://cytoscape.org/*, ver. 3.7.1).

There are some closely connected regions of molecular complexes in large PPI networks, which are called clusters or topology modules [30,31]. Using network topology attributes to integrate molecular functions and disease-related information, the hidden functional modules and disease information in protein networks might be discovered [31]. We identified the protein clusters using MCODE [30], a Cytoscape plug-in. Then, proteins contained in each cluster were entered into the STRING database to reproduce the corresponding cluster map and predict functional associations between proteins.

### Molecular docking

Molecular docking relies on the "lock-key principle" of ligand-receptor interaction and mimics the interaction between small molecule ligands and receptor bio-macromolecules [32]. Through calculations the combination mode and affinity between the compounds and proteins can be predicted [33,34]. The whole molecular docking process, performed on Molecular Operating Environment (MOE) (v2015.10), includes 3 steps: receptor preparation, receptor grid generation, and docking. Firstly, the crystallographic protein structure was downloaded from PDB bank (*https://www.wwpdb.org/*) and imported into MOE. Then, modified protein processing including protonation, water molecule removal, structure preparation, and energy minimization, was simulated. Subsequently, the receptor grid was generated for determining the docking site on proteins. The ligand position in the protein receptor was the site at which the docking was centered. Lastly, the 3-dimensional compound structure, drawn in ChemBioDraw, was imported into MOE, and standard docking was performed between the prepared protein structure and the 3-dimensional compound structure.

## Results

### ZJW compounds

Using databases, we identified a total of 234 compounds in ZJW (Supplementary Table 1), including 62 from R. coptidis and 180 from E. rutaecarpa. Based on the criteria (OB ≥30% and DL ≥0.18), 17 and 33 active compounds were identified from R. coptidis and E. rutaecarpa, respectively. Berberine, obacunone, and quercetin were the active ingredients shared by both herbs. The details of active compounds are described in Table 1.

### The putative target genes of ZJW

Based on the target prediction system in the TCMSP database, 176 and 172 putative targets were identified for R. coptidis and E. rutaecarpa, respectively. Most of the targets were overlapping, indicative of potential interactions between the 2 herbs in the course of treatment. Combining the targets of the 2 herbs, we obtained 188 putative ZJW gene targets (Supplementary Table 2).

### Identification of ZJW targets for gastric cancer treatment

Gastric cancer target genes were retrieved from GeneCards and OMIM. In total, 20 507 potential target genes related to gastric cancer were collected. By intersecting the 188 target genes of ZJW with the 20507 potential target genes related to gastric cancer, we obtained 185 intersections. These intersections were considered potential candidate targets of ZJW against gastric cancer. The vast majority of ZJW target genes overlapped, highlighting the efficacy of ZJW in gastric cancer treatment (Supplementary Table 3 and Figure 2A).

### Candidate therapeutic targets validation in TCGA database

A total of 2771 gastric cancer related DEmRNAs were identified in 381 tumor samples and 32 adjacent non-tumor samples from the TCGA database. These DEmRNAs included 1498 (54.06%) significantly upregulated genes and 1273 (45.94%) significantly downregulated genes (Supplementary Table 4).

Comparison of the 185 potential ZJW candidate targets against gastric cancer with the 2771 gastric cancer DEmRNAs from TCGA revealed 48 common genes. These common genes were considered potential anti-gastric cancer ZJW target genes (Table 2, Figure 2B).

### Network construction of putative ZJW targets

A network diagram consisting of 224 nodes and 550 edges was constructed using 47 active compounds and 188 ZJW target proteins (Figure 3). As shown in this compound-target network, nodes with a greater number or edges have greater importance within the network, are presented as larger in size, and are the ones on which to focus. The top 5 ingredient nodes with the greatest number of edges were quercetin, wogonin, baicalein, beta-sitosterol, and isorhamnetin. The top 5 gene nodes were *PTGS2*, *AR*, *PTGS1*, *SCN5A*, and *ADRB2*.





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**Table 1.** A list of the active compounds in Zuojinwan for network analysis.

| MOL ID | Compound name | Molecular formula | OB | DL |
|---|---|---|---|---|
| MOL001454 | Berberine | C20H18NO4+ | 36.86 | 0.78 |
| MOL013352 | Obacunone | C26H30O7 | 43.29 | 0.77 |
| MOL000098 | Quercetin | C15H10O7 | 46.43 | 0.28 |
| MOL002662 | Rutaecarpine | C18H13N3O | 40.3 | 0.6 |
| MOL000354 | Isorhamnetin | C16H12O7 | 49.6 | 0.31 |
| MOL000358 | Beta-sitosterol | C29H50O | 36.91 | 0.75 |
| MOL000359 | Sitosterol | C29H50O | 36.91 | 0.75 |
| MOL003942 | Rutaevine | C26H30O9 | 66.05 | 0.58 |
| MOL003943 | Rutalinidine | C15H17NO4 | 40.89 | 0.22 |
| MOL003947 | 1-methyl-2-[(Z)-pentadec-10-enyl]-4-quinolone | C25H37NO | 48.45 | 0.46 |
| MOL003950 | 1-methyl-2-[(Z)-undec-6-enyl]-4-quinolone | C21H29NO | 48.48 | 0.27 |
| MOL003956 | Dihydrorutaecarpine | C18H15N3O | 42.27 | 0.6 |
| MOL003957 | 1-methyl-2-pentadecyl-4-quinolone | C25H39NO | 44.52 | 0.46 |
| MOL003958 | Evodiamine | C19H17N3O | 86.02 | 0.64 |
| MOL003960 | 1-(5,7,8-trimethoxy-2,2-dimethylchromen-6-yl)ethanone | C16H20O5 | 30.39 | 0.18 |
| MOL003963 | Hydroxyevodiamine | C19H17N3O2 | 72.11 | 0.71 |
| MOL003964 | 1-methyl-2-undecyl-4-quinolone | C21H31NO | 47.59 | 0.27 |
| MOL003972 | 1-methyl-2-nonyl-4-quinolone | C19H27NO | 48.42 | 0.2 |
| MOL003974 | Evocarpine | C23H33NO | 48.66 | 0.36 |
| MOL003975 | Icosa-11,14,17-trienoic acid methyl ester | C21H36O2 | 44.81 | 0.23 |
| MOL003988 | 2-Hydroxy-3-formyl-7-methoxycarbazole | C14H11NO3 | 83.08 | 0.18 |
| MOL003994 | 24-methyl-31-norlanost-9(11)-enol | C30H52O | 38 | 0.75 |
| MOL004002 | 5alpha-O-(3'-methylamino-3'-phenylpropionyl)nicotaxine | C40H48N2O10 | 30.86 | 0.49 |
| MOL004004 | 6-OH-Luteolin | C15H10O7 | 46.93 | 0.28 |
| MOL004014 | Evodiamide | C19H21N3O | 73.77 | 0.28 |
| MOL004017 | Fordimine | C16H20N2O | 55.11 | 0.26 |
| MOL004018 | Goshuyuamide I | C19H19N3O | 83.19 | 0.39 |
| MOL004019 | Goshuyuamide II | C19H17N3O2 | 69.11 | 0.43 |
| MOL004020 | Gossypetin | C15H10O8 | 35 | 0.31 |
| MOL004021 | Gravacridoneshlirine | C19H18ClNO4 | 63.73 | 0.54 |
| MOL004025 | N-(2-methylaminobenzoyl)tryptamine | C18H19N3O | 56.96 | 0.26 |
| MOL004345 | 1-methyl-2-nonyl-4(1h)-quinolone | C39H67NO | 31.54 | 0.5 |
| MOL002419 | Higenamine | C16H17NO3 | 82.54 | 0.21 |
| MOL002894 | Berberrubine | C19H16ClNO4 | 35.74 | 0.73 |
| MOL002897 | Epiberberine | C20H18NO4+ | 43.09 | 0.78 |





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**Table 1 continued.** A list of the active compounds in Zuojinwan for network analysis.

| MOL ID | Compound name | Molecular formula | OB | DL |
|---|---|---|---|---|
| MOL002903 | (R)-Canadine | C20H21NO4 | 55.37 | 0.77 |
| MOL002904 | Berlambine | C20H17NO5 | 36.68 | 0.82 |
| MOL002907 | Corchoroside A_qt | C23H32O6 | 104.95 | 0.78 |
| MOL000622 | Magnograndiolide | C15H22O4 | 63.71 | 0.19 |
| MOL000762 | Palmidin A | C30H22O8 | 35.36 | 0.65 |
| MOL000785 | Palmatine | C21H22NO4+ | 64.6 | 0.65 |
| MOL001458 | Coptisine | C19H14NO4 | 30.67 | 0.86 |
| MOL002668 | Worenine | C20H16NO4+ | 45.83 | 0.87 |
| MOL008647 | Moupinamide | C18H19NO4 | 86.71 | 0.26 |
| MOL002714 | Baicalein | C15H10O5 | 33.52 | 0.21 |
| MOL002776 | Baicalin | C21H18O11 | 40.12 | 0.75 |
| MOL000173 | Wogonin | C16H12O5 | 30.68 | 0.23 |

OB – oral bioavailability; DL – drug-likeness.

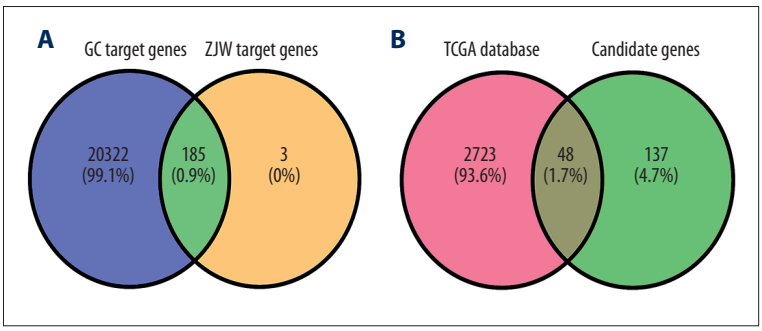


**Figure 2.** Zuojinwan (ZJW) target gene prediction for gastric cancer treatment. The overlapping number of gastric cancer (GC) and ZJW target genes (**A**), and the number of overlapping gastric cancer genes from The Cancer Genome Atlas (TCGA) database and ZJW candidate genes against GC (**B**).

### Network construction and enrichment analysis of ZJW anti- gastric cancer genes

After extracting information about the relationship between the 48 potential anti-gastric cancer target genes of ZJW from the STRING website, a gene-gene interaction network, containing 47 nodes and 190 edges, was constructed (Figure 4A). In the PPI network, the hub genes with the higher degree values were *MMP9*, *SERPINE1*, *CXCL8*, *MMP1*, *SPP1*, *ERBB2*, *CRP*, and *MMP3*. The number of edges connected to these nodes were relatively high (22, 19, 19, 18, 17, 16, 16, and 15 for *MMP9*, *SERPINE1*, *CXCL8*, *MMP1*, *SPP1*, *ERBB2*, *CRP*, and *MMP3*, respectively). Calculation results suggested that these central genes may be important or key target genes in the anti-gastric cancer effects of ZJW. MCODE network analysis revealed 4 clusters (Figure 4B, Table 3). The highest scoring cluster, cluster 1, contained 11 nodes and 53 edges, including *CXCL10*, *MPO*, *IFNG*, *MMP1*, *MMP9*, *SERPINE1*, *CRP*, *MMP3*, *SPP1*, *IL1A*, and *CXCL8*. Cluster 2 contained 5 nodes (*MAOB*, *ADRA1D*, *SLC6A4*, *ADRA1A*, and *SLC6A3*) and 53 edges. Cluster 3 and 4 each contained 3 nodes and 3 edges.

GO and KEGG pathway enrichment analyses were performed to explore the functions of the 48 potential anti-gastric cancer target genes of ZJW. In GO analysis, the 48 potential target genes were significantly enriched in 335 biological process (BP), 29 cell components (CC), and 49 molecular functions (MF) ($P<0.05$). The top 3 BP terms were blood circulation (GO: 0008015), circulatory system process (GO: 0003013), and response to xenobiotic stimulus (GO: 0009410) (Figure 5A). The top MF terms were mainly enriched in CXCR chemokine receptor binding (GO: 0045236), adrenergic receptor activity (GO: 0004935), and G protein-coupled amine receptor activity (GO: 0008227). The top CC terms included integral component of presynaptic membrane (GO: 0099056), intrinsic component of presynaptic membrane (GO: 0098889), and extracellular matrix (GO: 0031012). KEGG pathway analysis identified 19 pathways that potentially participate in the anti-gastric cancer





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**Table 2.** Target genes of Zuojinwan for treating gastric cancer.

| No. | Gene | No. | Gene | No. | Gene | No. | Gene |
|---|---|---|---|---|---|---|---|
| 1 | *COL1A1* | 13 | *TOP2A* | 25 | *KCNH2* | 37 | *MMP3* |
| 2 | *BIRC5* | 14 | *SLC2A4* | 26 | *E2F1* | 38 | *GSTM1* |
| 3 | *F7* | 15 | *CHRM2* | 27 | *CXCL11* | 39 | *ERBB2* |
| 4 | *CYP1A1* | 16 | *SERPINE1* | 28 | *CXCL8* | 40 | *PGR* |
| 5 | *CRP* | 17 | *PTGS1* | 29 | *MPO* | 41 | *CXCL10* |
| 6 | *ADRA1A* | 18 | *PYGM* | 30 | *ADRB2* | 42 | *DIO1* |
| 7 | *IL1A* | 19 | *AR* | 31 | *MGAM* | 43 | *GSTM2* |
| 8 | *SPP1* | 20 | *CYP3A4* | 32 | *COL3A1* | 44 | *SLC6A3* |
| 9 | *OLR1* | 21 | *TP63* | 33 | *ABCG2* | 45 | *PLAU* |
| 10 | *ADRA1D* | 22 | *GRIA2* | 34 | *MAOB* | 46 | *SLC6A4* |
| 11 | *MMP1* | 23 | *PRSS1* | 35 | *IGF2* | 47 | *PON1* |
| 12 | *ALOX12* | 24 | *MMP9* | 36 | *IFNG* | 48 | *CHEK1* |

mechanism of ZJW (Figure 5B). Among these pathways, "Bladder cancer", "IL-17 signaling pathway", and "Prostate cancer" were the most enriched. Additionally, our results also showed that platinum drug resistance, cellular senescence, and toll-like receptor signaling pathways were all closely connected to the progress and development of gastric cancer.

An interaction network was constructed using 57 active compounds and 48 potential anti-gastric cancer target genes of ZJW and a network diagram consisting of 84 nodes and 185 edges was obtained (Figure 6). The top 5 compound nodes with the greatest number of edges included quercetin, beta-sitosterol, isorhamnetin, wogonin, and baicalein. The top 5 gene nodes with the greatest number of edges included *PTGS1*, *AR*, *ADRB2*, *KCNH2*, and *PRSS1*.

Nineteen KEGG pathways, related target genes and corresponding compounds, were imported to Cytoscape to construct the active compound-target-pathway anti-gastric cancer network of ZJW, which contained 86 nodes and 227 edges (Figure 7). The "IL-17 signaling pathway" contained the highest number of genes (n=6) followed by "Platinum drug resistance" (n=5), and "Cellular senescence" (n=5). These pathways, with the highest number of potential targets, may be key pathways in ZJW treatment of gastric cancer. Interestingly, we found that some genes are involved in multiple pathways. For example, *CXCL8* is involved in both the "IL-17 signaling pathway" and the "Toll-like receptor signaling pathway". These results also show that ZJW acts on gastric cancer through multiple pathways, multiple targets, and overall cooperation.

## Molecular docking analysis

The core PPI network targets were input into the PDB database. Proteins were selected for MOE molecular docking analysis based on the published evidence, being from the human species, having a greater number of ligands, and being successfully able to build pockets in the MOE. Three key target proteins, including MMP9 (PDB code: 1gkc), MMP3 (PDB code: 1d5j), and MMP1 (PDB code: 1fbl), were selected as successful preparations of the receptor protein, followed by molecular docking with berberine, isorhamnetin, quercetin, wogonin, (R)-canadine, baicalein, baicalin, berlambine, and evodiamine.

MMP9 linked to berberine, isorhamnetin, quercetin, and wogonin (Figures 8A–8D). Berberine formed one hydrogen bond and one H-ϖ conjugation with GLU402 and LEU187 in MMP9, respectively (Figure 8a). Isorhamnetin formed 2 hydrogen bonds with GLU402 in MMP9 (Figure 8b). Quercetin formed one hydrogen bond and one H-ϖ conjugation with LEU188 and GLU402 in MMP9, respectively (Figure 8c). Wogonin formed one hydrogen bond with TYR423 in MMP9 (Figure 8d).

The binding modes of MMP3 with berberine, isorhamnetin, quercetin, and wogonin were determined (Figure 8E–8H). Berberine formed 2 π-H conjugations with ASN162 and VAL163 in MMP3 (Figure 8e). Isorhamnetin formed one H-π conjugation and one π-H conjugation with LEU164 and TYR155 in MMP3, respectively (Figure 8f). Quercetin formed one H-π conjugation and one π-H conjugation with LEU164 and TYR155 in MMP3, respectively (Figure 8g). Wogonin formed one π-π bond with HIS201 in MMP3 (Figure 8h).





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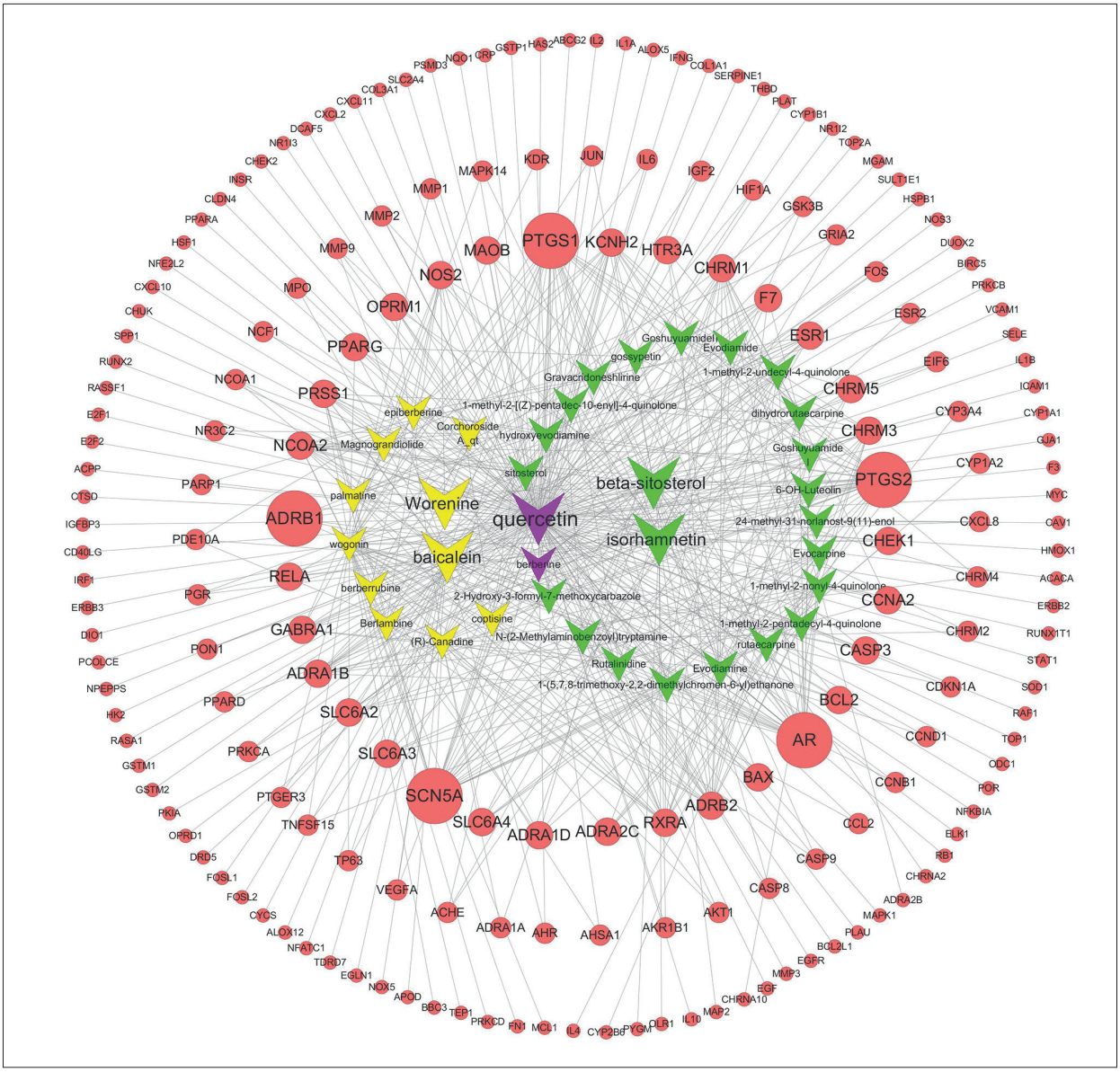


**Figure 3.** Compound-target network of Zuojinwan. Red circle nodes stand for the target genes. The representatives of yellow, green, and purple nodes stand for compounds in *R. coptidis*, *E. rutaecarpa* and both, respectively. The size of the node represents the size of the degree. *E. rutaecarpa* – *Euodia rutaecarpa*; *R. coptidis* – *Rhizoma coptidis*.

The binding modes of MMP1 with berberine, isorhamnetin, quercetin, and wogonin were determined (Figures 8I–8L). One π-H conjugation combined berberine with ASN180 in MMP1 (Figure 8i). Isorhamnetin was linked to GLU219 in MMP1 via 2 hydrogens (Figure 8j). Two hydrogens also combined quercetin with GLU219 in MMP1 (Figure 8k). Wogonin formed one hydrogen bond with GLU219 in MMP1, and 2 ϖ-H conjugations with LEU181 and TYR240 in MMP1 (Figure 8l).

In summary, docking simulation studies show that the different binding models between compounds and proteins have different binding abilities. The detailed energy docking score is described in Table 4. The lower the score, the more stable the ligand-receptor binding. The computational results indicate that representative compounds in ZJW could combine well with the target genes.

## Discussion

To the best of our knowledge, this is the first study integrating network pharmacology and molecular docking analyses to reveal the pharmacological mechanisms of ZJW for treating gastric cancer. We identified a total of 47 active compounds in ZJW.





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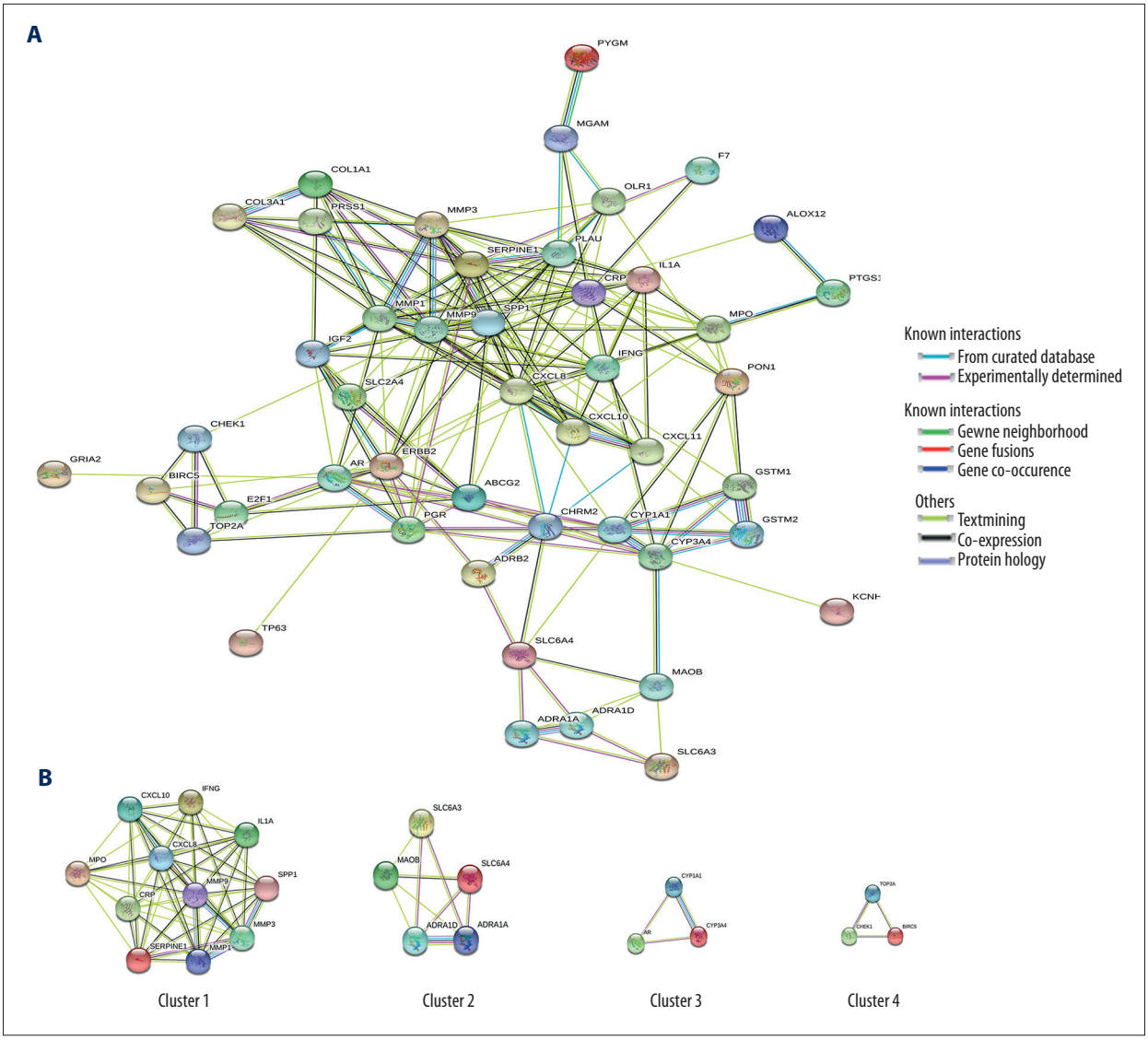


**Figure 4.** (**A, B**) Protein-protein interaction network of Zuojinwan anti-gastric cancer genes. Edges represent protein-protein associations, including known interaction (azure represents curated database evidences, purple represents experimentally determined evidences), predicted interactions (green represents gene neighborhood, red represents gene fusions, and blue represents gene co-occurrence), and others (light green represents text mining, black represents co-expression, and light blue represents protein homology).

**Table 3.** Cluster of target genes of Zuojinwan anti-gastric cancer protein-protein interaction network.

| Cluster | Score | Nodes | Edges | Node IDs |
|---|---|---|---|---|
| 1 | 10.6 | 11 | 53 | *CXCL10, MPO, IFNG, MMP1, MMP9, SERPINE1, CRP, MMP3, SPP1, IL1A, CXCL8* |
| 2 | 4.5 | 5 | 9 | *MAOB, ADRA1D, SLC6A4, ADRA1A, SLC6A3* |
| 3 | 3 | 3 | 3 | *CYP1A1, CYP3A4, AR* |
| 4 | 3 | 3 | 3 | *TOP2A, CHEK1, BIRC5* |





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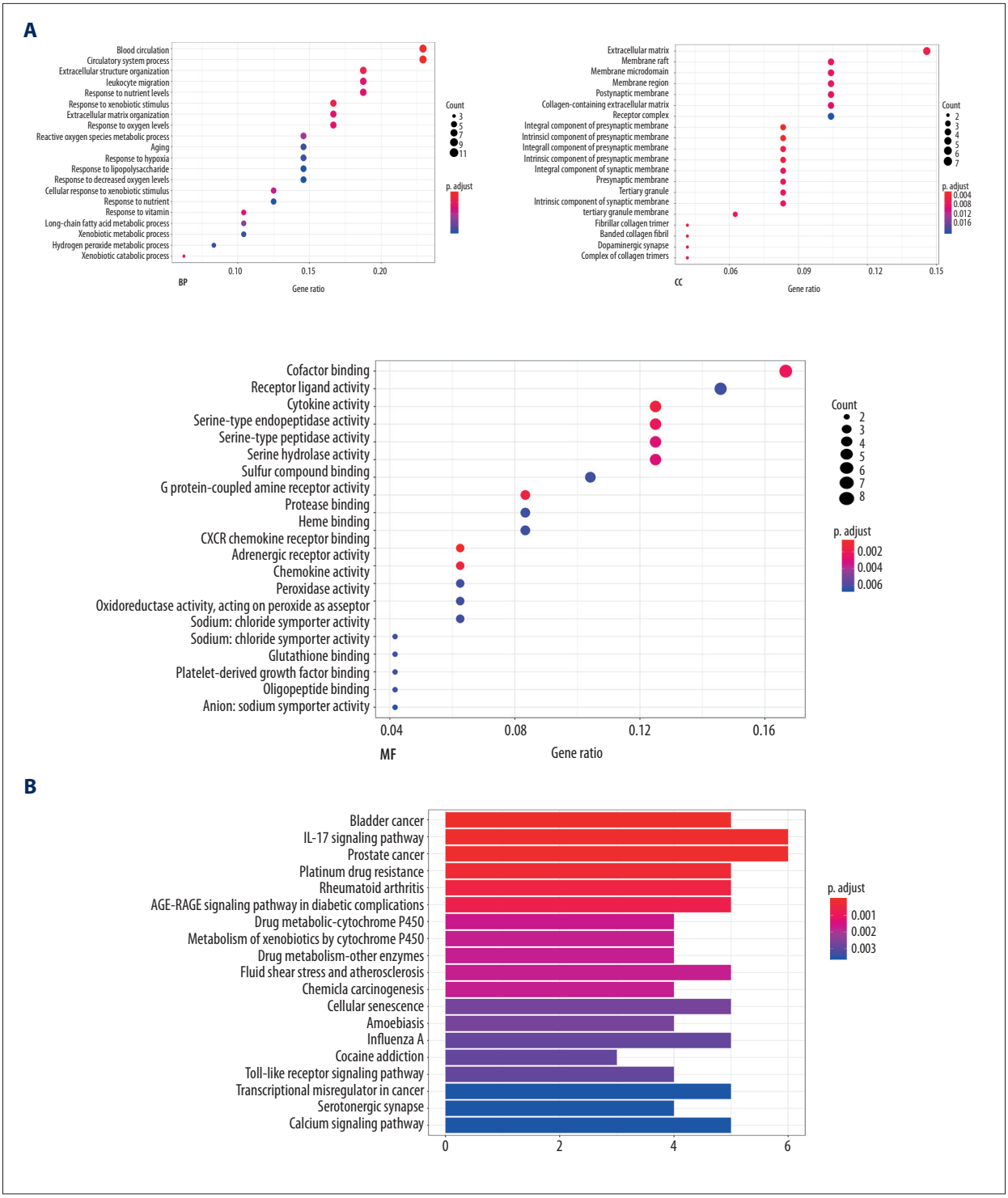


**Figure 5.** GO and KEGG enrichment analysis of Zuojinwan anti-gastric cancer genes. The GO enrichment analysis of Zuojinwan anti-gastric cancer genes (**A**). The ontology covered 3 domains: biological process, cellular component, and molecular function. The abscissa GeneRatio represents the proportion of genes of interest in the entry, and the ordinate represents each entry. The size of the dots represents the number of genes annotated in the entry, and the color of the dots represents the corrected *P*-value of the hypergeometric test. The KEGG enrichment analysis of Zuojinwan anti-gastric cancer genes (**B**). The abscissa represents the number of genes annotated in the pathway, the ordinate represents the pathway, and the color of the column represents the corrected *P*-value. KEGG – Kyoto Encyclopedia of Genes and Genomes; GO – Gene Ontology.





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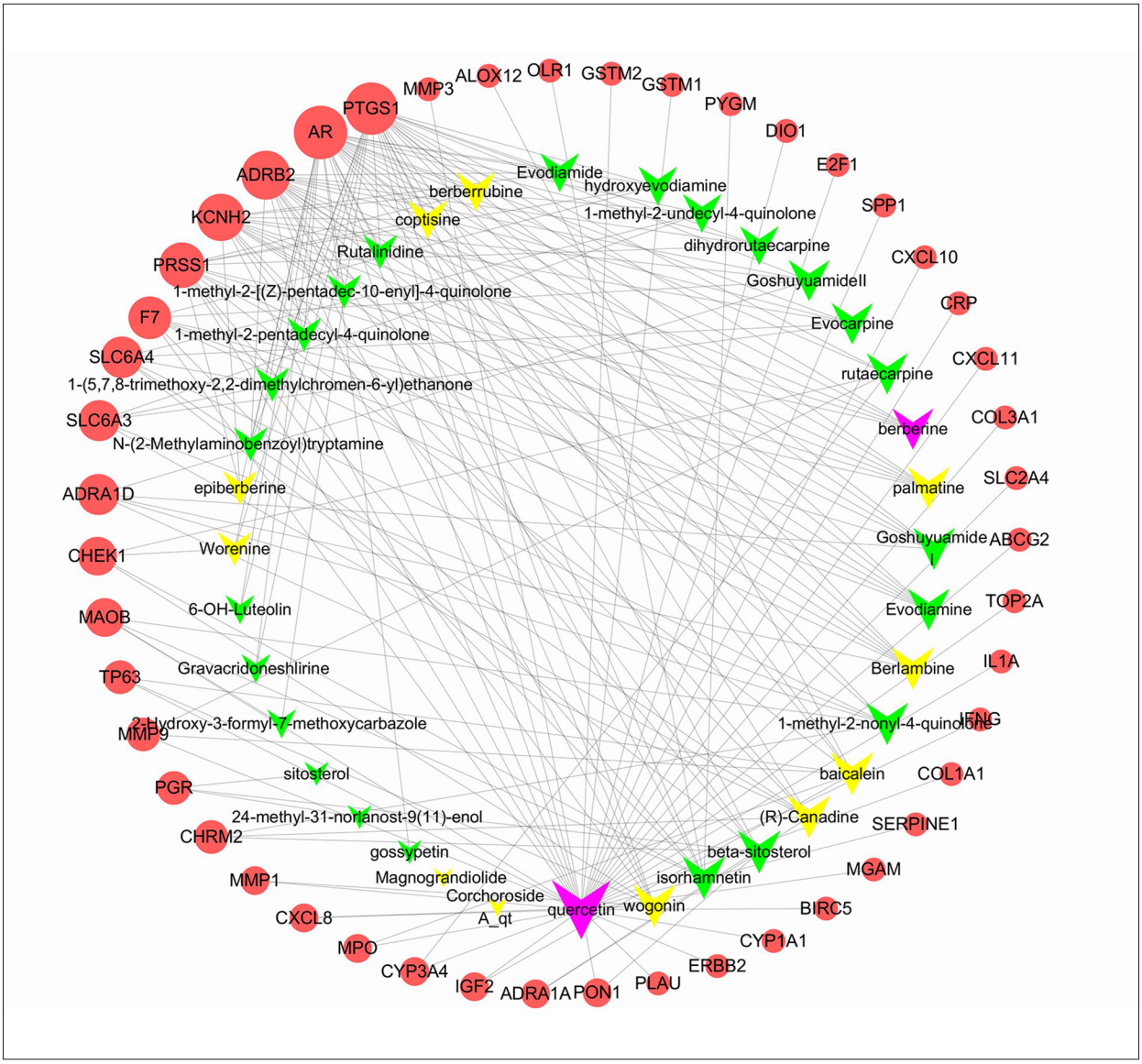


**Figure 6.** Potential compounds-potential targets network of Zuojinwan for treating gastric cancer. The representative of red, orange, yellow, and purple nodes is the same as Figure 3.

Furthermore, a total of 48 potential target genes related to the action of ZJW in gastric cancer were identified. PPI analysis revealed that the top-ranking genes, *MMP9*, *SERPINE1*, *CXCL8*, *MMP1*, *SPP1*, *ERBB2*, *CRP*, and *MMP3*, may be the crucial targets for treating gastric cancer. Functional enrichment analysis revealed the over-represented GO terms and their functional domains. KEGG pathway enrichment analysis revealed that the 48 target proteins were significantly enriched in 19 related signal pathways. Among them, interleukin (IL)-17 signaling and platinum drug resistance appeared to be the most critical pathways involved in the treatment of gastric cancer. Furthermore, molecular docking analysis demonstrated that the representative compounds could bind to the target protein binding site.

In recent years, numerous natural products originating from Chinese herbal medicine have attracted considerable attention as anti-cancer agents because of their high therapeutic value and low systemic toxicity [35]. Previous studies have demonstrated that berberine and evodiamine, the major bioactive ingredients in ZJW, exhibit anti-cancer effects in gastric cancer by inhibiting proliferation and inducing apoptosis and autophagic cell death [36–39]. Other representative ingredients in ZJW, including baicalein, baicalin, wogonin, isorhamnetin, and beta-sitosterol, are also involved in the anti-gastric cancer effect [40–44]. Collectively, a series of studies involving the active ZJW ingredients have provided evidence for the efficacy of ZJW in the treatment of gastric cancer. By constructing a component-target relationship network, we showed for





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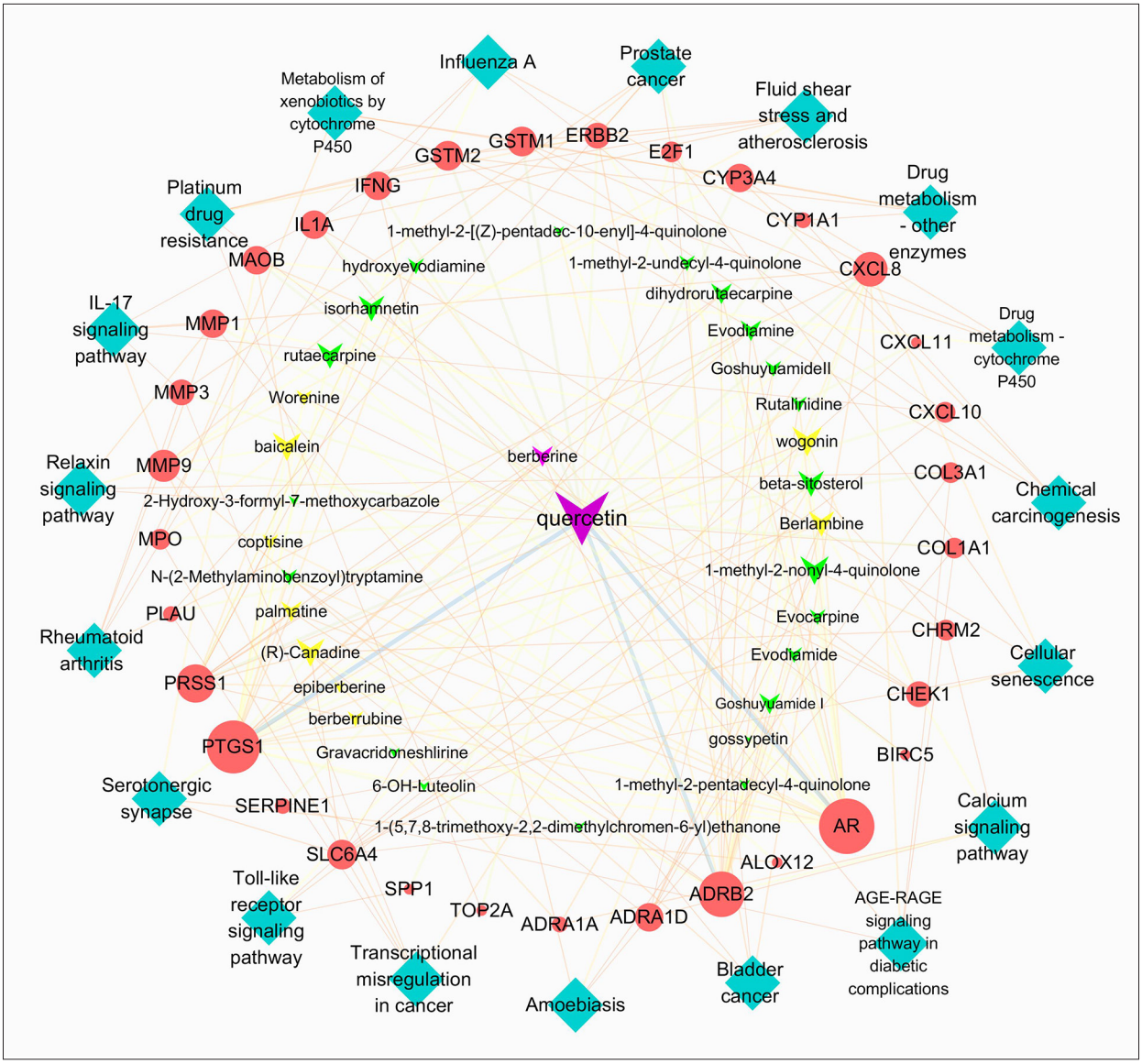


**Figure 7.** Potential compounds-potential targets-pathways network of Zuojinwan for treating gastric cancer. Blue diamond nodes stand for pathways. The representative of red, orange, yellow, and purple nodes is the same as Figure 3.

the first time that quercetin is the most important ZJW compound for treating gastric cancer. Quercetin is a polyphenolic compound with many biological activities and is abundantly distributed in plants, foods, and beverages [45]. In a large Swedish population-based case-control study, it was observed that quercetin significant negatively correlates with the risk of noncardiac gastric adenocarcinoma [46]. Quercetin not only has its own cytotoxic effect on gastric cancer cells, but also acts synergistically with cytostatic drugs (such as daunorubicin). Therefore, quercetin is considered to be a prospective chemosensitizer with the ability to overcome resistance to chemotherapy drugs in gastric cancer cells [47,48]. These observations further highlight that quercetin plays a vital role in the function of ZJW in gastric cancer.

Matrix metalloproteinases (MMPs), endopeptidases with diverse biochemical functions, possess the ability to promote cancer cell invasion and metastases formation [49]. Interestingly, 3 core PPI network genes, *MMP9*, *MMP3*, and *MMP1*, belong to the MMP family. Several genetic polymorphisms have been identified that show allele specific effects on MMP9 regulation and are associated with gastric cancer [50]. Increased production and activation of MMP3 and MMP1 might promote remodeling of the cell microenvironment in gastric cancer [51]. These data suggest that the effect of ZJW on gastric cancer occurs through some MMP family genes. Hence, the molecular docking method was used to explore the *in-silico* simulation matching between these genes and the representative compounds in ZJW. Molecular docking results show that MMP9, MMP3, and





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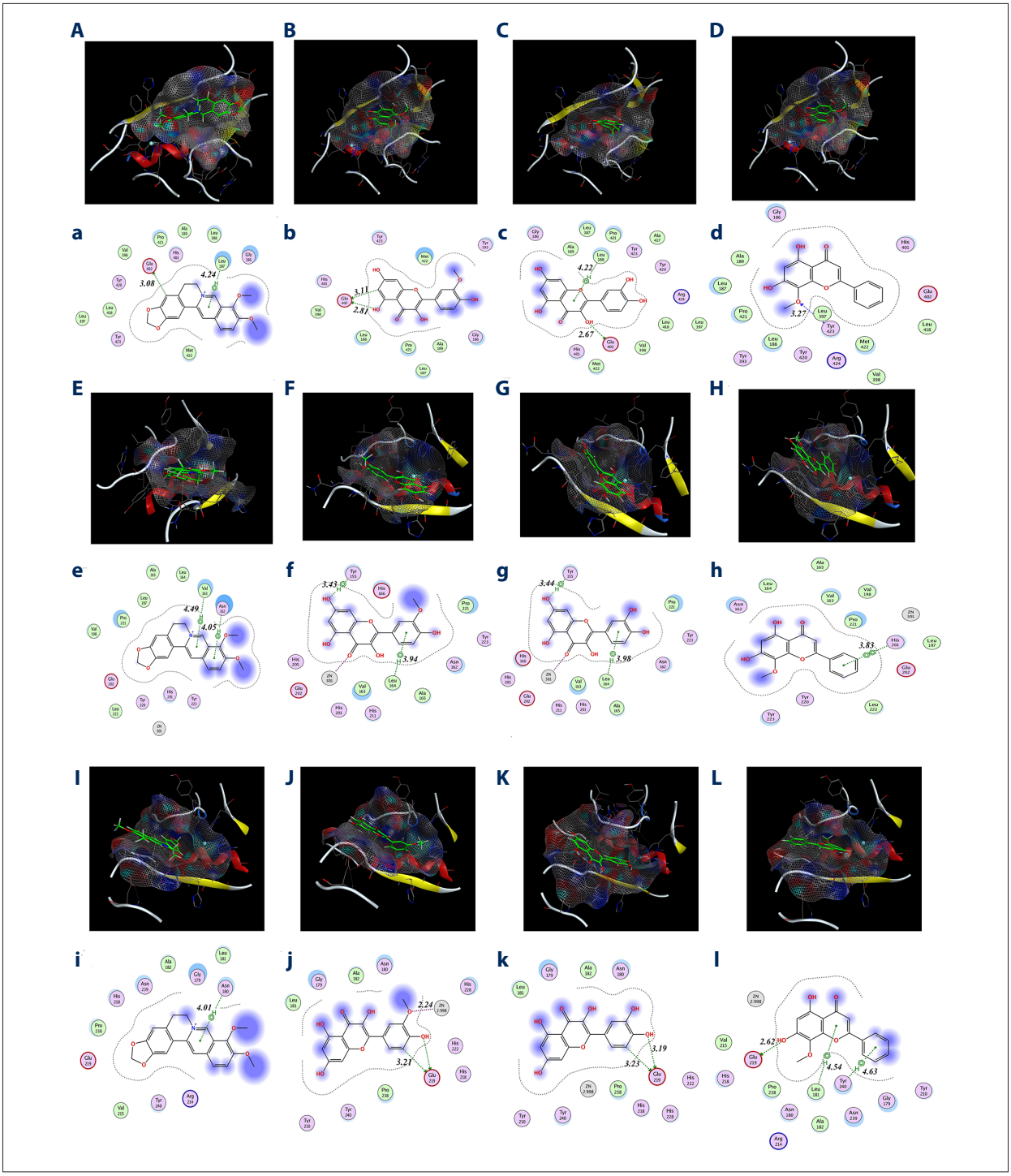


**Figure 8.** The docking model of compounds with MMP9, MMP3, and MMP1. Binding model of berberine (**A**), isorhamnetin (**B**), quercetin (**C**), and wogonin (**D**) on the molecular surface of MMP9. The interaction model of berberine (**a**), isorhamnetin (**b**), quercetin (**c**), and wogonin (**d**) with MMP9. Binding model of berberine (**E**), isorhamnetin (**F**), quercetin (**G**), and wogo0nin (**H**) on the molecular surface of MMP3. The interaction model of berberine (**e**), isorhamnetin (**f**), quercetin (**g**), and wogonin (**h**) with MMP3. Binding model of berberine (**I**), isorhamnetin (**J**), quercetin (**K**), and wogonin (**L**) on the molecular surface of MMP1. The interaction model of berberine (**i**), isorhamnetin (**j**), quercetin (**k**), and wogonin (**l**) with MMP1. The ligands in binding model and interaction model are colored in green and within the dashed line, respectively. The length of the bond was added on the bond.





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**Table 4.** Virtual docking of 4 representative ingredients from Zuojinwan for gastric cancer targets.

| Compound | Binding energy/(kcal·mol$^{-1}$) | | |
|---|---|---|---|
| | MMP9 | MMP3 | MMP1 |
| Original ligands | –7.9091 | –11.8849 | –14.5777 |
| Berberine | –5.8617 | –5.6970 | –5.7199 |
| Isorhamnetin | –5.8996 | –7.3793 | –7.1915 |
| Quercetin | –6.1569 | –7.2459 | –7.1467 |
| Wogonin | –6.4024 | –5.7839 | –5.9911 |
| (R)-Canadine | –5.1318 | –6.2006 | –5.6461 |
| Baicalein | –4.9929 | –5.7758 | –6.3437 |
| Baicalin | –7.5559 | –7.0784 | –7.4166 |
| Berlambine | –5.5981 | –7.0967 | –5.5062 |
| Evodiamine | –5.1372 | –6.1891 | –5.3646 |

MMP – matrix metalloproteinases.

MMP1 exhibited good affinity for those compounds, indicating an interactive relationship between the active compounds and target genes. Further research is required to confirm these results. Other core genes are closely connected to the anti-gastric cancer effect. A clinical study has shown that *CXCL8* is strongly associated with worse overall survival in patients with gastric cancer and may be a potential biomarker for gastric cancer [52]. Silencing of *SPP1* may also contribute to the inhibition of proliferation, migration, invasion, and the epithelial-to-mesenchymal transition in gastric cancer cells via suppression of the PI3K/AKT signaling pathway [53].

KEGG pathway analysis revealed that ZJW-targeted genes were mainly associated with signal pathways diseases, especially tumor-related disease. This suggests that ZJW might exert anti-tumor effects on various malignant tumors. Furthermore, we found that ZJW exerts a therapeutic effect on gastric cancer through multiple pathways. Among them, IL-17 signaling and platinum drug resistance are key pathways that may be involved in the anti-gastric cancer mechanism of ZJW. IL-17, a CD4 T cell-derived mediator of angiogenesis, plays a major role in stimulating angiogenesis. Constitutive activation of IL-17A regulates cancer cell proliferation, migration, and apoptosis, and induces the elaboration of other protumor factors *in vitro* [54]. In addition, the IL-17 signaling pathway plays a pivotal role in the development of gastric cancer through multiple pathways, including inflammation and immunity [55]. Our network analysis revealed that the IL-17 signaling pathway contained the most genes. Additional enriched pathways involved in gastric cancer pathology include "platinum drug resistance". Previous studies indicate that ZJW extracts can enhance the proapoptotic effects of DDP in human gastric cancer SGC-7901/DDP cells and the inhibiting effects of DDP on tumor growth in an SGC-7901/DDP xenograft model [11]. Taken together, these data suggest that ZJW might be used as a synergistic drug with chemotherapeutic drugs for treating gastric cancer, although further research should be conducted to confirm this effect.

Here, we used network pharmacology and data analysis to elucidate the therapeutic mechanism of ZJW in gastric cancer, and validated the results using molecular docking analysis. The network pharmacological analysis approach applied still had several limitations, including the need for further pharmacological experiments to validate the anti-gastric cancer mechanisms of ZJW.

## Conclusions

Our results have uncovered the multi-component, multi-target, and multi-pathway potential mechanism underlying the action of ZJW in gastric cancer. These results enhance our understanding of the potential anti-tumor effects of ZJW in gastric cancer. Moreover, the target genes and pathways most likely to be associated with the potential anti-gastric cancer mechanisms of ZJW are worthy of further experimental validation.

### Data availability

The data used to support the findings of this study are available from the corresponding author upon request, unless there are legal or ethical reasons for not doing so.

### Conflicts of interest

None.





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## Supplemenatry Data

**Supplementary Table 1.** A total of 234 compounds in ZJW retrieved from databases.
**Supplementary Table 2.** A list of potential target genes of Zuojinwan.
**Supplementary Table 3.** Potential candidate targets of Zuojinwan for treating gastric cancer.
**Supplementary Table 4.** A total of 2771 DEmRNAs related to gastric cancer found in the TCGA database.

Supplementary tables available from the corresponding author on request.

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