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Study on the Mechanism of Jiedu Sanjie Formula in the Treatment of Gastric Cancer Based on Network Pharmacology and Molecular Docking

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Abstract

Objective: This study aimed to investigate the potential active ingredients, core targets and molecular mechanisms of Jiedu Sanjie Formula in the treatment of gastric cancer, thereby providing a theoretical basis for its clinical application and experimental research. **Methods:** The effective ingredients and corresponding targets of Jiedu Sanjie Formula were screened using TCMSP, TCMID and HERB databases, as well as network resources, including GeenMedical and Chinese literature. Gastric cancer-related targets were retrieved from DrugBank, OMIM, TTD and GeneCards databases. With STRING platform, the protein-protein interaction network was established, from which core targets were identified. GO functional enrichment and KEGG pathway enrichment analyses were performed using the DAVID database, and the pathway-target network diagram was visualized with Gephi 0.9.2. Finally, molecular docking was conducted with AutoDock Tool-1.5.7, and the results were visualized by PyMOL software. **Results:** A total of 38 active ingredients were identified in Jiedu Sanjie Formula, corresponding to 859 drug-related targets, among which 497 overlapped with gastric cancer targets. The main active ingredients included quercetin, stigmasterol, cinobufagin, and cinobufotalin. Mechanistically, the formula exerted anti-gastric cancer effects by acting on core targets such as AKT1, AKT3, MAP2K1, PIK3R1 and PIK3CD, thereby regulating tumor-related signaling pathways including the PI3K-Akt pathway, HIF-1 pathway and cancer-related pathways. Molecular docking results confirmed good binding affinity between core active ingredients and key targets. **Conclusion:** Based on network pharmacology prediction, Jiedu Sanjie Formula can synergistically mediate the pathological processes such as proliferation and apoptosis of gastric cancer cells through multi-component, multi-target and multi-pathway modes. Its anti-gastric cancer mechanism is closely related to the regulation of classical tumor signaling pathways, providing theoretical support for subsequent basic experimental validation and clinical translation.

1 Introduction

Gastric cancer is a malignant tumor arising from gastric mucosa epithelial cells and is one of the digestive system tumors with a high mortality worldwide. According to the latest global cancer epidemiology statistics, Asia bears the highest disease burden; in China, gastric cancer ranks fifth in incidence and third in mortality among all malignancies, imposign heavy social and medical costs [1]. Despite relative complete clinical therapies for gastric cancer, including intervention regimens such as surgical resection, chemotherapy, radiotherapy, targeted therapy and immunotherapy, clinical efficacy is often undermined by low response rates, acquired cancer resistance, severe toxicity and a high postoperative long-term recurrence rate [2].

Recent research has demonstrated that traditional Chinese medicine (TCM) has unique advantages in modulating cancer immune microenvironment, suppressing gastric cancer cell proliferation and invasion, enhancing antic-cancer immune responses in the body and alleviating chemotherapy-related side effects [3]. According to TCM theory, the fundamental nature of gastric cancer is deficiency in the root and excess in branch [4]. In early stages, liver qi stagnation predominates; with disease progression, the healthy qi gradually declines. Meanwhile, pathogenic toxin, turbid phlegm, blood stasis, and heat toxicity accumulate and stagnate together, finally forming a complicated state of intermingled deficiency and excess with intractable pathology [5]. Thus, the core treatment principle of reinforcing healthy qi and supplementing qi, detoxifying and dissipating masses, and eliminating pathogenic factors to resolve accumulation must be maintained throughout all clinical stages.

Jiedu Sanjie Formula is a prescription developed for gastric cancer pathogenesis featuring phlegm, toxin and blood stasis intermingling. It consists of *Paridis Rhizoma* (eliminating heat-toxin, relieving swelling and pain, cooling blood and resolving stagnant masses) [6], *Bufonis Corium* (dried toad skin; clearing heat and removing toxicity, transforming phlegm and softening hard masses, and activating blood and resolving masses) [7], *Prunella vulgaris* (clearing liver and purging fire, and dissipating masses and reducing swelling) [8], and *Citri Reticulatae Pericarpium* (regulating qi to remove stagnation, unblocking qi movement, promoting blood circulation and fluid distribution, and resolving phlegm and stasis) [9]. The combined use of multiple Chinese medicinal herbs can exert synergistic effects, including dissipating stagnation and accumulation, clearing cancer toxins and fortifying spleen and stomach [10,11]. Thus, the formula acts as a promising adjuvant for gastric precancerous lesions as well as for the middle and advanced gastric cancer, through improving the symptoms and overall conditions of patients. Nevertheless, modern molecular mechanism through which the formula intervenes in gastric cancer has been poorly studied. Specifically, its effects on cancer cell proliferation, apoptosis and key cancer signaling pathways have yet to be systematically elucidated. To bridge this knowledge gap, the present study employed network pharmacology and molecular docking technologies to comprehensively analyze the multi-ingredient, multi-target, and multi-pathway mechanisms of Jiedu Sanjie Formula against gastric cancer, thereby providing a reliable theoretical basis and scientific evidence for its experimental validation and standardized clinical promotion and application of this formula.

2 Methods

2.1 Active ingredients and target screening of TCM

Jiedu Sanjie Formula consisted of *Bufonis Corium* (dried toad skin), *Prunella vulgaris, Citri Reticulatae Pericarpium* (tangerine peel), and *Paridis Rhizoma* (rhizome of Paris polyphylla). The ingredients of

Prunella vulgaris and *Citri Reticulatae Pericarpium* were retrieved from TCMSP (https://www.tcmsp-e.com/) and TCMID (http://www.megabionet.org/tcmid/) databases, using the criteria OB ≥ 30% and DL ≥ 0.18. The ingredients of *Bufonis Corium* and *Paridis Rhizoma* were obtained from GeenMedical, Chinese literature [12,13], and HERB (http://herb.ac.cn/). After the intersection of the results from multiple sources for each Chinese medicinal herb, 25 active ingredients were identified from *Bufonis Corium* and 13 from *Paridis Rhizoma*. The two-dimensional structures and SMILES notations of the active chemical ingredients of *Bufonis Corium* and *Paridis Rhizoma* were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Compounds lacking corresponding chemical structures or SMILES notations were excluded, as their associated targets could not be retrieved, resulting in 12 active compounds from *Bufonis Corium* and 10 from *Paridis Rhizoma* for subsequent network pharmacology analysis. The potential targets of the obtained compounds were then queried using the TCMID database [14-16], the Swiss-Target database (http://www.swisstargetprediction.ch/), and the STITCH database (http://stitch.embl.de/).

2.2 Screening of disease targets

Gastric cancer-related targets were screened from DrugBank, OMIM, TTD, and GeneCards databases using the key word "gastric cancer".

2.3 Prediction of TCM–Disease Potential Targets

The predicted targets of the active ingredients in TCM and the gastric cancer targets were imported into Venny 2.1.0 to generate a Venn diagram. The overlapping targets were identified as potential therapeutic targets.

2.4 Establishment of the Protein–Protein Interaction (PPI) Network

The potential targets were uploaded into the STRING database (https://www.string-db.org/), with the species set as Homo sapiens and a confidence score threshold of ≥ 0.9 [15]. The data were exported in TSV format and subsequently imported into Cytoscape 3.8.2 software to generate a PPI network diagram.

2.5 Biological Function Analysis and Pathway Analysis of Cancer Targets

After deduplication, the intersection of TCM-derived targets and gastric cancer-related targets was obtained using Venny to identify potential therapeutic targets. These targets were then input into the DAVID database (version 6.8, https://david.ncifcrf.gov/) for Gene Ontology (GO) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. The species was set to Homo sapiens, and the default enrichment threshold (EASE score ≤ 0.1) was applied. GO terms and KEGG pathways with a false discovery rate (FDR)-corrected *P*-value < 0.05 were considered statistically significant.

2.6 TCM–Ingredient–Target Network Diagram

The data for TCM ingredients and their corresponding targets (overlapping targets) were imported into Gephi 0.9.2 to construct a TCM – ingredient – target network diagram.

2.7 Molecular Docking

Molecular docking was conducted to validate the interactions between the top 10 TCM effective ingredients and top 6 core pathway targets. Concretely, 3D structures of small molecule ligands were downloaded from TCMSP and PubChem databases, and then all hydrogen atoms were added. The molecules were set as the ligand (with automatic assignment of charges). After the rotatable bonds were detected and set, the structures were exported in PDBQT format. The 3D crystal structures of the core proteins were obtained from the PDB database. These structures were processed by removing water

molecules, excess ligands, and heteroatoms, followed by addition of all hydrogen atoms, and then set as the receptor and exported in PDBQT format.

Later, spatial docking parameters were configured in AutoDock Tools 1.5.7 to generate the docking run configuration file. Blind docking was performed using a uniform grid box size of 126 × 126 × 126A to ensure complete coverage of the entire protein surface for all target proteins. The specific grid center coordinates (x, y, z) for each ligand-target pair were provided in Table S1.

Semi-flexible molecular docking simulations were performed using the AutoDock Vina program, with the binding free energy (ΔG) serving as the core indicator of binding affinity between the ligand and receptor. A lower binding energy is indicative of a stronger spontaneous binding ability between the ingredient and the target, as well as a more stable conformation of the complex. Finally, the 3D visualization models illustrating the interactions between the core ingredients and key targets were generated and exported using PyMOL software.

3 Results

3.1 Acquisition of Active Ingredients and Targets of Jiedu Sanjie Formula

The 11 active ingredients from *Prunella vulgaris* and 5 from *Citri Reticulata Pericarpium* were retrieved from TCMSP database in accordance with defined screening criteria. The 12 active ingredients of *Bufonis Corium* and 10 of *Paridis Rhizome* were ultimately obtained by initially supplementing active ingredients of the two herbs from HERB database, relevant literature and pharmacological studies, and later removing compounds without corresponding standard chemical structures in PubChem database. All the above active ingredients were individually imported into Swiss-Target, STITCH and TCMID databases to predict potential targets. Target names were subsequently converted to official gene symbols using the Uniport database(https://www.uniprot.org/). A total of 4,107 original TCM-related target records were obtained by removing 144 duplicate targets. The compiled targets were input into the Uniport database for official gene symbol standardization, with the species restricted to Homo sapiens. Following the elimination of non-human targets, targets without corresponding coding genes, duplicate entries, and redundant invalid targets, 859 effective targets of Jiedu Sanjie Formula were ultimately identified.

3.2 Acquisition of Gastric Cancer-related Targets

Gastric Cancer-related targets were retrieved from four databases: TTD (http://db.idrblab.net/ttd/), GeneCards (https://www.genecards.org/), DrugBank (https://go.drugbank.com/), and OMIM (https://omim.org/). A total of 3,989 disease targets were obtained (Figure 1).

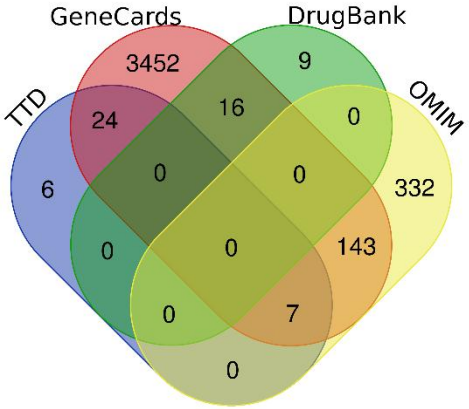

Figure 1 Disease targets.

3.3 Intersection of TCM Targets and Disease Targets

The predicted TCM targets and disease targets were uploaded into the Venny 2.1.0 online analysis platform (http://bioinformatics.psb.ugent.be/) to generate a Venn diagram illustrating the TCM-disease target intersection. A total of 497 potential common targets of the Jiedu Sanjie Formula against gastric cancer were obtained by extracting the overlap between the two sets, which were subsequently used for network construction and functional enrichment analysis (Figure 2).

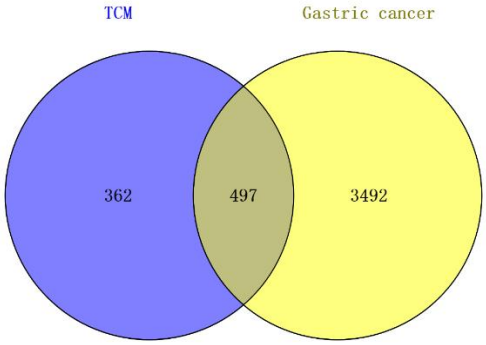

Figure 2 Venn diagram of the intersection of Jiedu Sanjie Formula active targets and gastric cancer targets.

3.4 PPI Network

The 497 potential TCM-disease intersecting targets were imported into the STRING 12.0 database. The species was set to Homo sapiens and the confidence score threshold was set to ≥ 0.9 [15]. The results were exported in TSV format and then imported into Cytoscape 3.8.2 software to construct a visualized PPI network. Nodes with low connectivity (degree value ≤ 20) were removed from the network. By topological analysis of the network, SRC, MAPK1, TP53, PIK3R1, HSP90AA1, STAT3, AKT1, PIK3CA, and GRB2 were identified as the top 10 hub targets with the highest degree values, implying their central roles in the regulatory mechanism of the Jiedu Sanjie Formula against gastric cancer. These top 10 hub targets constituted the first-tier screening pool. In the subsequent pathway-target network analysis, targets with high pathway-specific connectivity will be further refined from this pool to identify core regulatory nodes for molecular docking validation (Figure 3).

3.5 Analysis of Gene Ontology (GO) Biological Function and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway

The 497 intersecting targets were analyzed using the DAVID database for GO functional and KEGG pathway enrichment [17]. A total of 1,141 biological process (BP) terms, 125 cellular component (CC) terms, 252 molecular function (MF) terms, and 142 KEGG signaling pathway terms were enriched. Sorted in ascending order by P-value, the top 10 most significantly enriched terms from BP, CC, and MF were selected to generate GO enrichment bubble plots (Figure 4 and 5).

GO analysis revealed that at the biological process level, targets were significantly enriched in core tumor-associated processes including protein phosphorylation, negative regulation of apoptotic

process, and positive regulation of cell proliferation. At the cellular component level, the target proteins were mainly localized to the cytoplasm, cell membrane, and plasma membrane complex. At the molecular function level, the targets were primarily enriched in ATP binding, protein kinase activity, and protein serine/threonine kinase activity.

KEGG analysis indicated that the core pathways of the Jiedu Sanjie Formula in the treatment of gastric cancer were predominantly concentrated in classic tumor-related regulatory pathways, including Pathways in Cancer, PI3K-Akt signaling pathway, and HIF-1 signaling pathway. A higher rich factor indicates greater enrichment significance and stronger pathway association.

3.6 TCM-Ingredient-Target Network Analysis

The active ingredients of the Jiedu Sanjie Formula and the intersecting target data were input into Gephi 0.9.2 software to construct a visualized "TCM-ingredient-target" interaction network. Topological analysis of the network yielded two sets of top 10 key nodes: key active ingredients with the highest connectivity including quercetin, kaempferol, luteolin, beta-sitosterol, nobiletin, β -ecdysone, stigmasterol, cinobufagin, morin, and cinobufotalin; and core targets with the highest degree values in the network containing BACE1, PTPN1, PTGS1, CYP19A1, HSD11B1, KDR, ADORA1, PTGS2, MDM2, and CA1 (Figure 6).

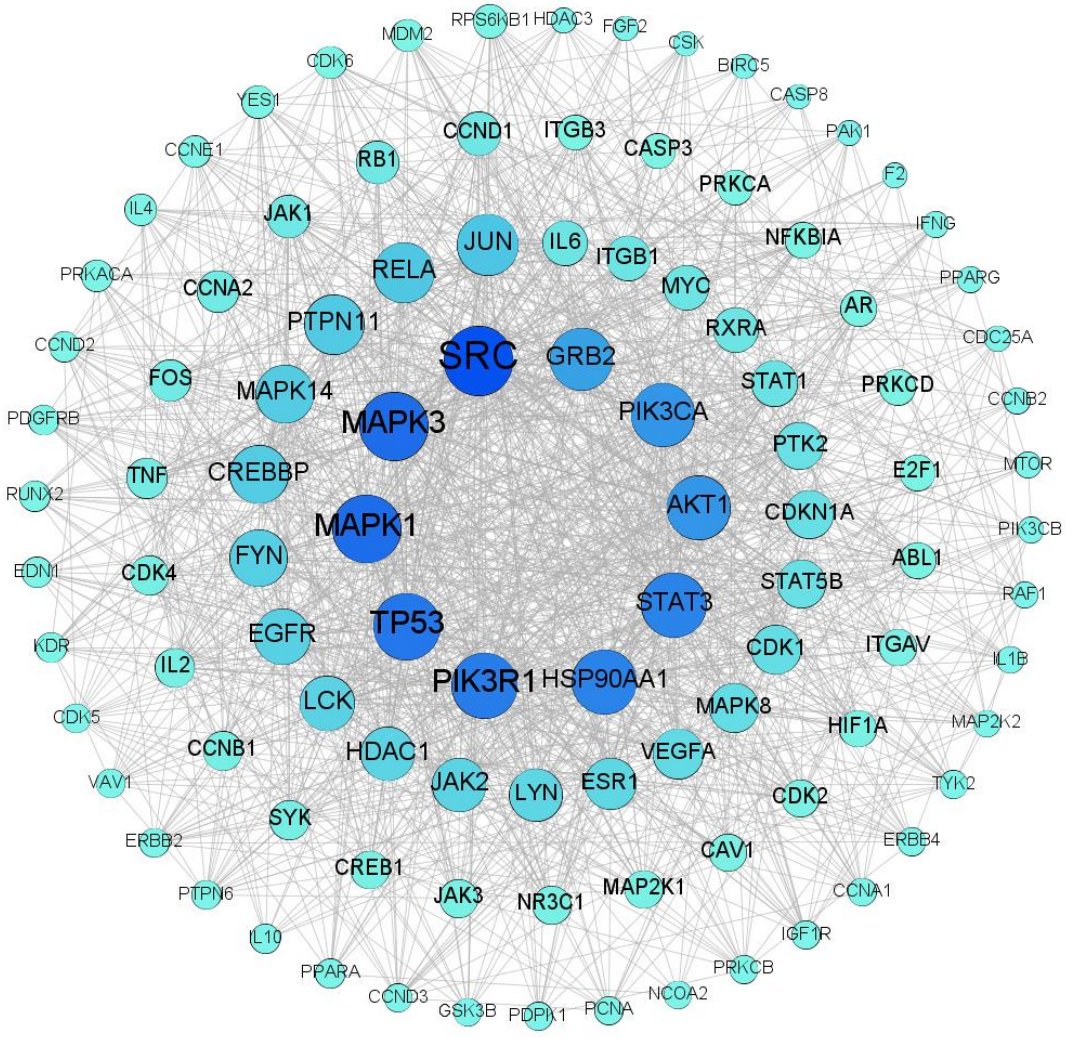

Figure 3 PPI network of Jiedu Sanjie Formula targets against gastric cancer.

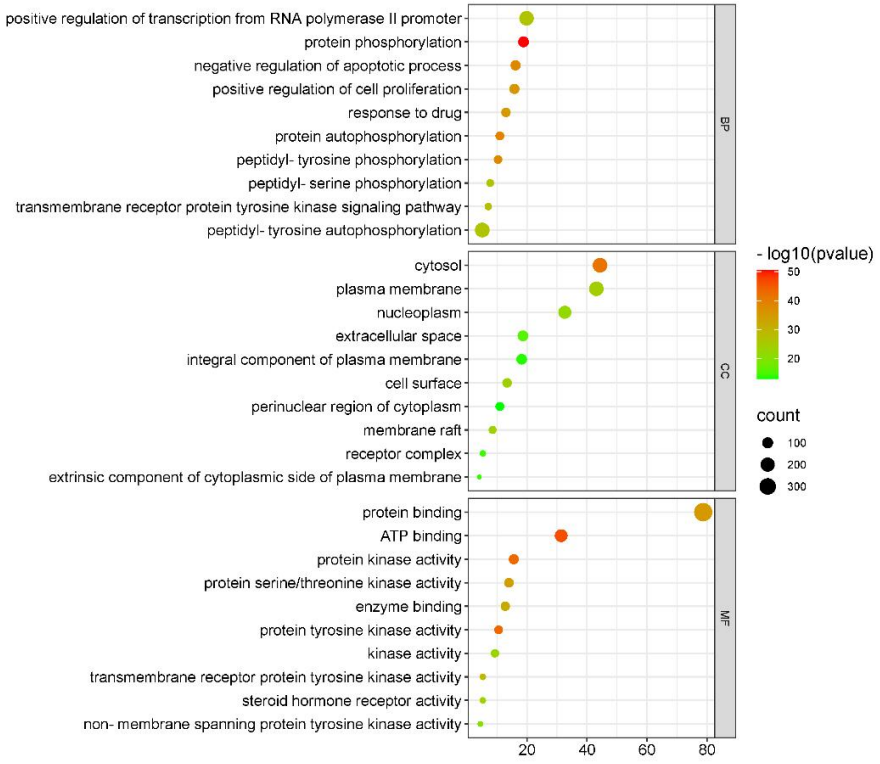

Figure 4 Bar plots of GO functions (BP, CC, MF) of Jiedu Sanjie Formula against gastric cancer.

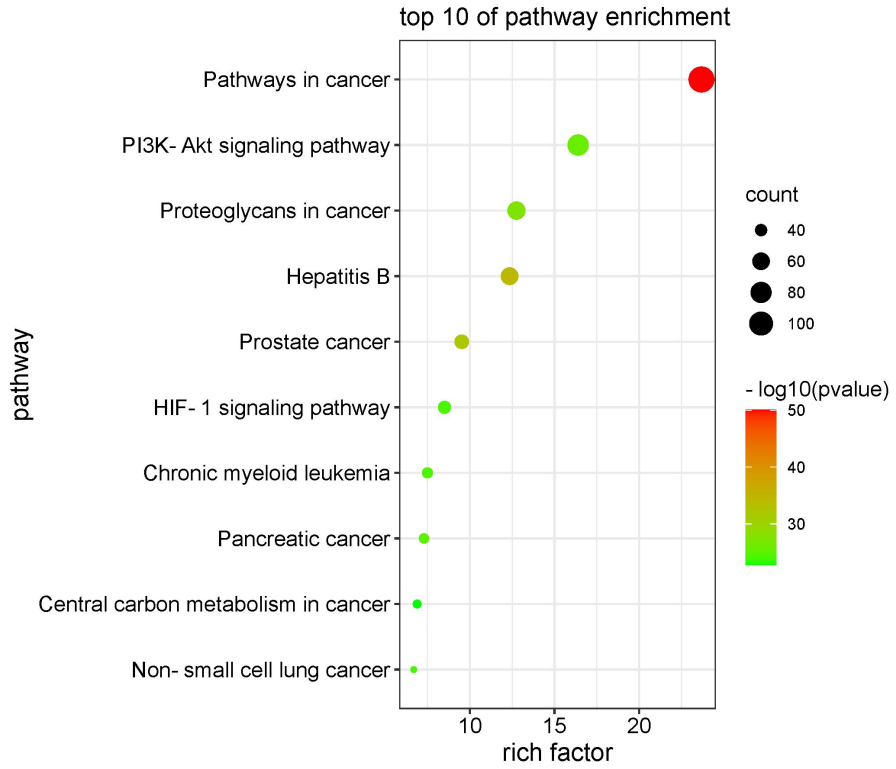

Figure 5 Top 10 KEGG pathways of Jiedu Sanjie Formula against gastric cancer.

3.7 Diagram of Pathway-Target Analysis

To identify the second-tier core targets, the top 10 KEGG pathways and their associated targets were uploaded into Gephi 0.9.2 software for pathway-target interaction network visualization. After the removal of isolated nodes with low connectivity (degree value <1) from the network, the top 10 core regulatory targets of the pathways were identified (see Figure 7). These targets, together with the PPI hub targets from Section 3.4, formed the basis for final docking target selection.

3.8 Molecular docking

The final six docking targets (AKT1, AKT2, AKT3, MAP2K1, PIK3R1, PIK3CD) were selected from the intersection of Tier 1 (PPI hub targets) and Tier 2 (pathway-target core targets). The AKT family was prioritized, because AKT1 ranked among the top PPI hub targets, all three isoforms were central nodes in the pathway-target network, and they served as the principal convergence point of the PI3K-Akt pathway (the most significantly enriched KEGG pathway). Together with the upstream PI3K subunits (PIK3R1, PIK3CD) and the MAPK node MAP2K1, these targets formed a coherent upstream-downstream regulatory axis for mechanistic validation. The key active ingredients, including quercetin, kaempferol, luteolin, beta-sitosterol, nobiletin, β-ecdysone, stigmasterol, cinobufagin, morin, and cinobufotalin, were subjected to molecular docking with the top six core pathway targets, using AutoDockTools-1.5.7 software to evaluate their binding affinities. According to Table 1 (binding energy results), stable and efficient interactions between the active components and target proteins were observed at the molecular level.

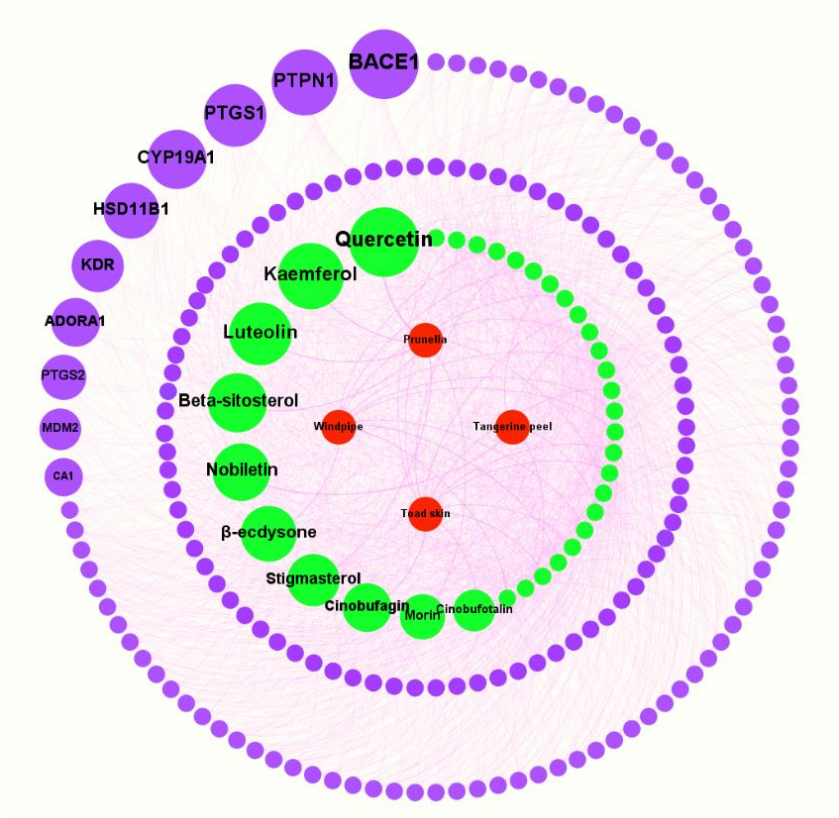

Figure 6 TCM-ingredient-target network of the Jiedu Sanjie Formula against gastric cancer.

Note: Red nodes represent the four Chinese medicinal herbs comprising the Jiedu Sanjie Formula; green nodes represent the corresponding active ingredients; purple nodes represent the corresponding targets.

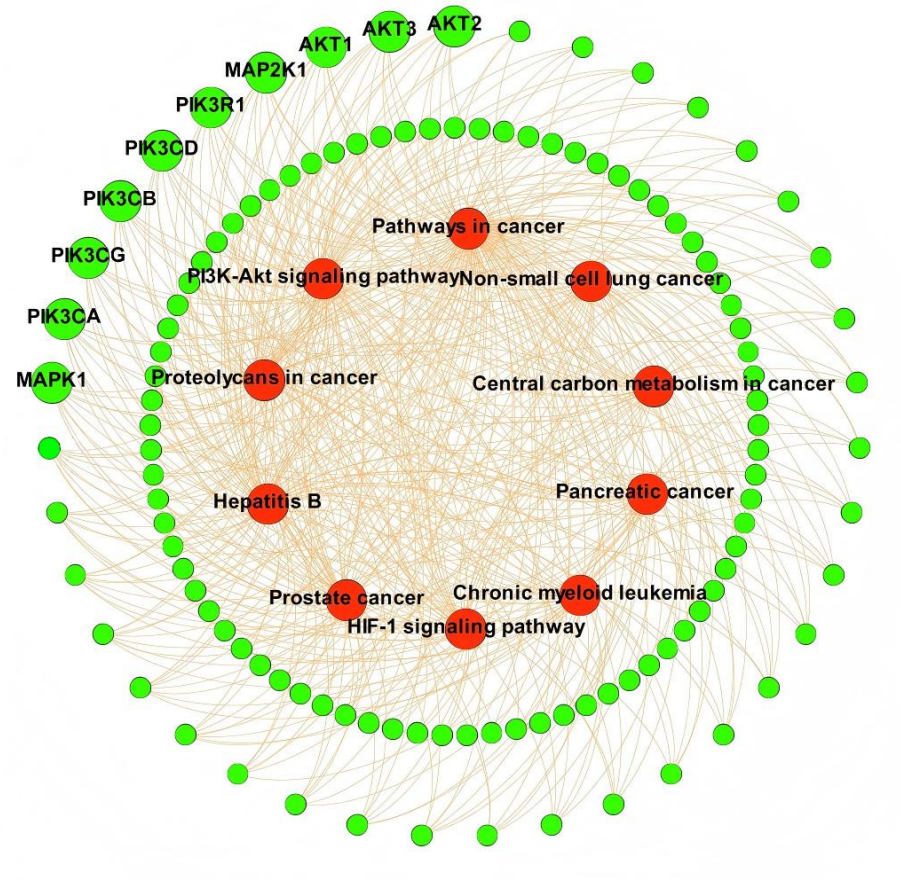

Figure 7 Pathway-target network of the Jiedu Sanjie Formula against gastric cancer.

Note: Red nodes indicate the names of the top 10 selected pathways; green nodes indicate the corresponding genes of the pathways.

The results showed that the binding energies of most active ingredients to the target proteins were ≤ -5.0 kcal·mol^{-1}, indicating favorable binding affinities [18]. Among them, ingredients such as stigmasterol and cinobufagin exhibited relatively low binding energies with targets including AKT1, MAP2K1, AKT3, and PIK3R1, suggesting that they may regulate gastric cancer-related signaling pathways through multi-target synergistic effects. Meanwhile, targets such as AKT1 and AKT3 demonstrated strong binding affinities with multiple active components, further highlighting their critical roles in the anti-gastric cancer mechanism of the Jiedu Sanjie Formula.

Based on the molecular docking results, a heatmap of binding energies between the top 10 core active ingredients of the Jiedu Sanjie Formula and the top 6 key targets (AKT2, AKT3, AKT1, MAP2K1, PIK3R1, PIK3CD) was generated (see Figure 8). In the heatmap, a darker color indicates a lower binding energy between active components and target proteins, representing stronger binding affinity.

The six ingredient–target pairs with the best docking results were imported into PyMOL for 3D visualization [19] (see Figure 9).

The above results intuitively demonstrated the "multi-ingredient, multi-target" characteristics of the Jiedu Sanjie Formula, providing direction for subsequent *in vitro* cellular experiments and mechanistic validation.

Table 1 Molecular docking results of core active ingredients of the Jiedu Sanjie Formula with key targets.

No	Active ingredient	Binding energy / (kcal- mol^{-1})					
		AKT2	AKT3	AKT1	MAP2K1	PIK3R1	PIK3CD
1	Quercetin	-6.74	-6.35	-7.01	-7.71	-5.85	-6.22
2	Kaempferol	-6.83	-5.97	-6.82	-7.53	-6.18	-5.08
3	Luteolin	-7.14	-6.97	-7.16	-8.03	-6.52	-5.55
4	Beta-sitosterol	-8.77	-8.25	-9.08	-8.46	-7.95	-7.45
5	Nobiletin	-5.78	-5.88	-7.85	-6.76	-7.23	-5.19
6	β-ecdysone	-5.78	-7.81	-7.53	-8.21	-7.4	-4.21
7	Stigmasterol	-7.74	-9.1	-10.89	-9.24	-8.15	-7.91
8	Cinobufagin	-7.93	-8.31	-9.99	-8.57	-8.82	-6.3
9	Morin	-6.39	-5.75	-6.98	-7.73	-6.08	-5.45
10	Cinobufotalin	-7.32	-7.05	-8.5	-7.28	-7.47	-6.04

Note: In this study, molecular docking was performed between the top 10 core active ingredients of the Jiedu Sanjie Formula and 6 key target proteins, namely AKT2, AKT3, AKT1, MAP2K1, PIK3R1, and PIK3CD. The 3D crystal structures of AKT2 (PDB ID: 3D0E), AKT1 (PDB ID: 3O96), MAP2K1 (PDB ID: 8YP4), PIK3R1 (PDB ID: 1H9O), and PIK3CD (PDB ID: 6OCO) were downloaded from the RCSB PDB database (https://www.rcsb.org/). Since no resolved crystal structure was available for AKT3, its predicted structure was retrieved from AlphaFold (UniProt ID: Q9Y243, Model ID: AF-Q9Y243-F1). The global pLDDT score for this model is 82.07, indicating high overall confidence. Notably, the pLDDT score at residue position 271, which is located within the ATP-binding active site region directly relevant to ligand docking, is 90.88. This value falls within the "very high confidence" category (pLDDT > 90), confirming exceptional structural reliability in the functionally critical binding pocket. The binding energies were presented in kcal·mol^{-1}. A lower binding energy corresponded to a stronger binding affinity between the active ingredient and the target protein, with values ≤ -5.0 kcal·mol^{-1} indicative of a favorable binding affinity.

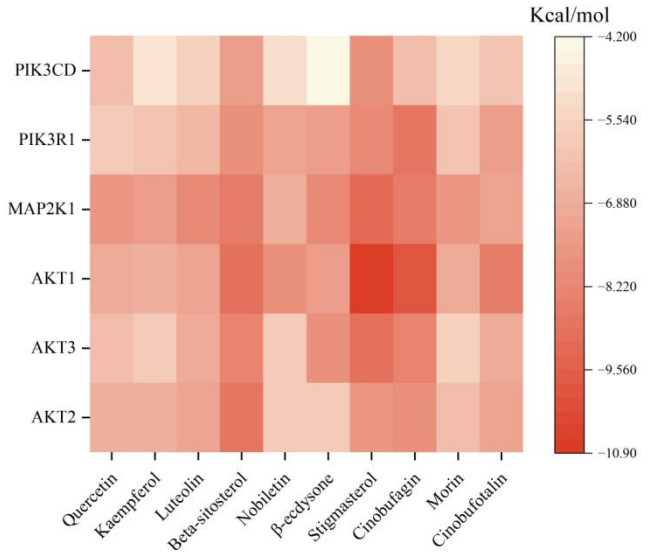

Figure 8 Heatmap of binding energies between core active ingredients of the Jiedu Sanjie Formula and key targets.

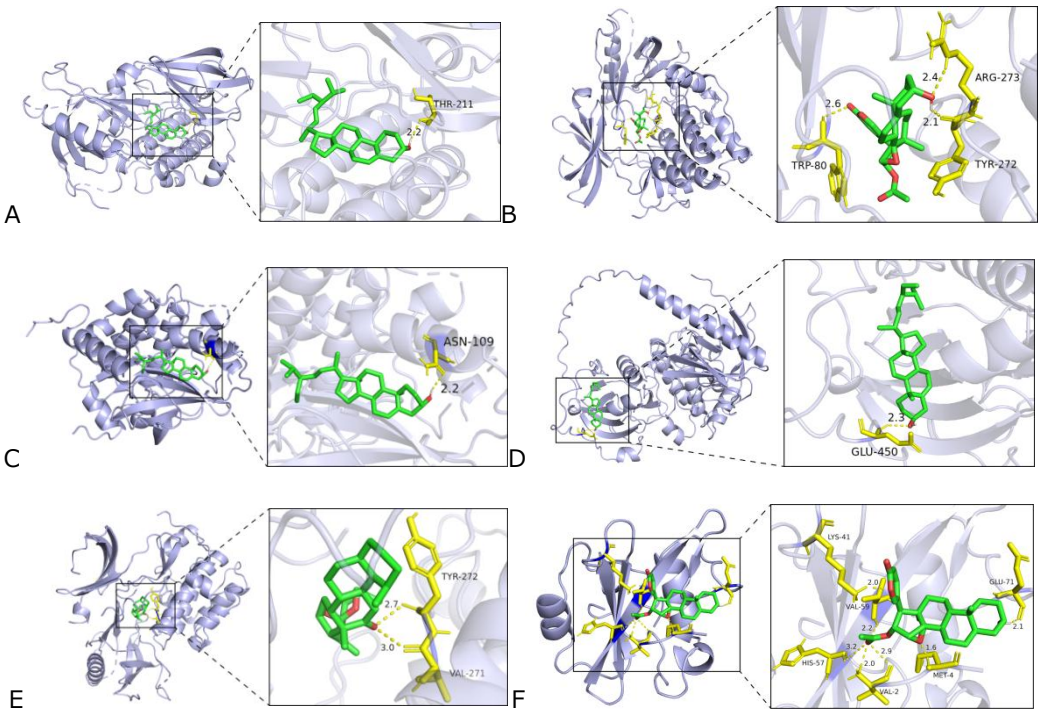

Figure 9 Visualization of molecular docking between core active ingredients of the Jiedu Sanjie Formula and key targets.

Note: Panels A-F show the docking results of core ingredient-target pairs ranked by binding energy (lowest to highest): A: Stigmasterol-AKT1 (-10.89 kcal/mol), B: Cinobufagin-AKT1 (-9.99 kcal/mol), C: Stigmasterol-MAP2K1 (-9.24 kcal/mol), D: Stigmasterol-AKT3 (-9.1 kcal/mol), E: β-sitosterol-AKT2 (-9.08 kcal/mol), and F: Cinobufagin-PIK3R1 (-8.82 kcal/mol).The gray-blue cartoons denote the 3D conformation of the target protein, the green structure represents the small molecule of the active ingredient, and the yellow portions indicate hydrogen bonds and binding sites.

4 Discussion

Gastric cancer is a frequently-occurring malignant tumor in the digestive tract worldwide, with its incidence and mortality consistently ranking among the highest of all tumors, imposing heavy burdens on patients [20]. In this study, we systematically investigated the core active substances of Jiedu Sanjie Formula against gastric cancer using network pharmacology technology. After screening, multiple key effective ingredients were obtained, including quercetin, kaempferol, luteolin, beta-sitosterol, nobiletin, β -ecdysone, stigmasterol, cinobufagin, morin, and cinobufotalin. Among them, quercetin, a flavonoid compound, can repress gastric cancer cell cycle, induce apoptosis, and suppress cell proliferation by reducing cell telomerase activity and enhancing chemosensitivity via inhibition of PI3K/AKT pathway [21-23]. Kaempferol, also a natural flavonoid compound, significantly suppresses gastric cancer cell proliferation *in vitro*, induces G2/M phase arrest and promotes cell apoptosis through mitochondria. Its core mechanism involves inactivation of the PI3K/Akt and ERK/MAPK signaling pathways, as well as modulation of cell cycle- and apoptosis-related protein expression levels. *In vivo* experiments have also demonstrated that kaempferol inhibits the growth of gastric cancer xenografts with low toxicity, establishing it as an important natural active monomer for anti-gastric cancer therapy [24]. Luteolin exerts anti-gastric cancer effects through suppressing PI3K/Akt signaling pathway. Specifically, it downregulates phosphorylated Akt level, and

modulates protein expression of Bax/Bcl-2, thereby inhibiting gastric cancer cell proliferation and inducing apoptosis [25]. Beta-sitosterol is a natural plant sterol widely distributing in vegetable oils, plant seeds and nuts, characterized by low toxicity and anti-cancer activity. Beta-sitosterol can upregulate p-ERK1/2 and Bcl-2 in cells, modulate the Ras/Raf/MAPK signaling cascade within an appropriate concentration range, and enhance immune-related effector expressions as well as cytotoxic activity against gastric cancer cells [26]. Stigmasterol, an active sterol monomer in the formula, inhibits gastric cancer cell proliferation and migration, induces blockage at G2/M stage and mitochondrial apoptosis, and exerts its anti-cancer effects through Akt/mTOR and JAK/STAT pathways [27,28]. Cinobufagin, a steroidal tropane-like active monomer derived from toad, can induce gastric cancer cell death and reverse chemoresistance by targeting HIF-1α, AKT/mTOR and other pathways [29-31]. Moreover, multiple active ingredients, such as nobiletin, β-ecdysone, morin, and cinobufotalin, act synergistically with the aforementioned main active ingredients, constituting the complex material basis for the anti-gastric cancer effects of Jiedu Sanjie Formula.

Beyond these core pathway-target interactions, the ingredient-target network identified additional high-connectivity targets including BACE1, PTPN1, PTGS1, CYP19A1, HSD11B1, KDR, ADORA1, PTGS2, MDM2, and CA1. These targets exemplified the formula's polypharmacological breadth, namely, the capacity of multiple ingredients to engage diverse protein targets simultaneously. Although these targets are not central to the PI3K-Akt-MAPK axis that dominated our mechanistic interpretation (either due to a lack of established roles in gastric cancer pathogenesis or due to their extensively characterization in prior studies), some may still contribute auxiliary effects. For instance, PTGS2-mediated prostaglandin synthesis [32] and KDR-driven angiogenesis [33] may complement the core anti-proliferative mechanism in the tumor microenvironment, warranting future stroma-focused investigations.

After PPI network construction and topological analysis, we observed that node degrees of targets such as SRC, MAPK1, MAPK3, TP53, AKT1, and PIK3R1 were significantly higher than those of other proteins. These proteins were located at the core hub of network, serving as the key regulatory nodes that mediated the effects of the formula's active ingredients on gastric cancer progression.

GO functional and KEGG pathway enrichment analyses demonstrated that the anti-gastric cancer effects of Jiedu Sanjie Formula mainly involved biological processes, including modulation of cell proliferation and apoptosis, protein phosphorylation, kinase signaling transduction, etc. The targets were mainly distributed in cell membrane and cytoplasm, and exerted their functions primarily by binding with proteins and modulating kinase activities. KEGG results showed that the core targets were remarkably enriched in Pathways in Cancer and the PI3K-AKT signaling pathway, as well as the HIF-1 pathway, proteoglycans in cancer, and other classical pro-tumor pathways, collectively regulating the malignant progression of gastric cancer through multiple signaling pathways. These findings implied that Jiedu Sanjie Formula did not rely on a single target, but rather exerted its anti-gastric cancer effects through the synergistic multi-pathway modulation.

By constructing pathway-target interaction network and screening top 10 core targets, this study ultimately identified AKT2, AKT3, AKT1, MAP2K1, PIK3R1, and PIK3CD as key targets. Classical mechanistic studies have demonstrated that downstream AKT family molecules could be phosphorylated and activated by PI3K compound upon activation. With this axis, PIK3R1 (encoding

PI3K pathway regulatory subunit) and PIK3CD (encoding its catalytic subunit) together constituted the upstream functional core in PI3K/AKT signaling axis, while AKT1, AKT2, and AKT3, the three major isoforms of AKT, served as the key downstream effector molecules. In addition, complicated pathway crosstalk and intersection modulation exist between AKT signaling pathway and MAP2K1-mediated MAPK pro-cancer pathway. These two pathways collectively promote abnormal proliferation, malignant invasion and chemoresistance of gastric cancer cells through a concerted signaling cascade [34,35]. The core targets screened in this study, including PIK3R1, PIK3CD, AKT1/2/3, and MAP2K1, were fully consistent with the upstream – downstream regulatory logic and molecular mechanisms of the classical PI3K-AKT and MAPK pro-tumor pathways, further validating the reliability and scientific rigor of the network prediction results.

The results of molecular docking have further verified the binding affinity between the core ingredients of Jiedu Sanjie Formula and the key targets. Ingredients such as stigmasterol, cinobufagin, Beta-sitosterol, and cinobufotalin exhibited strong binding affinity to targets including AKT2, AKT3, AKT1, MAP2K1, PIK3R1, and PIK3CD. Notably, six ingredient–target pairs, stigmasterol-AKT1, cinobufagin-AKT1, stigmasterol-MAP2K1, stigmasterol-AKT3, β-ecdysone-AKT2, and cinobufagin-PIK3R1, demonstrated excellent binding affinity, with binding energies all ≤ -8.82 kcal·mol^{-1}, and formed stable protein conformations via hydrogen bonds. These findings suggested that the Jiedu Sanjie Formula exerted its anti-cancer effects by intervening in these key targets and blocking pro-tumor signal transduction. Noteworthily, while PIK3CA represents the canonical oncogenic driver in gastric cancer epithelial cells [36], our selection of PIK3CD for molecular docking deliberately targets the immune-modulatory dimension of Jiedu Sanjie Formula. Emerging evidence has unveiled that PIK3CD-mediated signaling in tumor-infiltrating lymphocytes and myeloid cells contributes to immune evasion and stromal remodeling in gastric cancer [37]. By targeting PIK3CD alongside PIK3R1, our docking strategy identified a PI3K isoform with distinct tissue specificity, potentially illuminating the formula's effects on tumor immune microenvironment — a mechanism that complemented its direct cytotoxic action on cancer cells.

Notably, network pharmacology and molecular docking have become increasingly standardized methodologies in TCM research. However, the present study distinguished itself through three distinctive contributions: (1) application novelty: this study is the first to systematically elucidate the anti-gastric cancer mechanism of Jiedu Sanjie Formula, transforming clinical empirical application into verifiable molecular hypotheses; (2) analytical stringency: the adoption of high-confidence thresholds (STRING $\geq$ 0.9, degree $\geq$ 20) and multi-database cross-validation enhances the reliability of predictions beyond routine analyses; and (3) translational directionality: identification of the AKT-PIK3R1-MAP2K1 axis as the core regulatory hub establishes a prioritized research framework for subsequent experimental validation, biomarker development, and clinical stratification of gastric cancer patients who may optimally benefit from Jiedu Sanjie Formula intervention.

The rationale underlying this study stems from three potential gaps in the current literature. First, while classical formulas such as Huanglian Jiedu Decoction and Xiaojianzhong Decoction have been relatively well characterized through network pharmacology approaches, empirical formulas created for specific syndrome patterns, particularly those containing toxic animal-derived medicines, remain insufficiently explored at the mechanistic level, which may limit their integration into evidence-based clinical

frameworks. Second, although the PI3K/Akt pathway is hyperactivated in approximately 30–40% of gastric cancers, the majority of existing network pharmacology studies have focused on PIK3CA in epithelial cells, with comparatively fewer investigations exploring the immune-modulatory PIK3CD isoform as a potential therapeutic target in the tumor microenvironment. Third, an emerging trend suggested that network pharmacology studies could benefit from moving beyond primarily descriptive enrichment analyses toward more prescriptive prioritization strategies — identifying not only the pathways potentially implicated, but also the specific component-target interactions that merit prioritized experimental validation. This study represents an exploratory step toward addressing these considerations.

However, there are some limitations in this study. For example, it neither included in-depth investigation on compatibility principles of formulas and "Jun-Chen-Zuo-Shi" compatibility principle (also known as the monarch-minister-assistant-guide principle), nor involved validation analysis using clinical samples. Through *in vitro* experiments and *in vivo* experiments, future studies should investigate the effects of the core active ingredients and key regulatory targets screened herein on gastric cancer cell proliferation, apoptosis, invasion, and migration, and validate the regulatory mechanisms of the core pathways. Concurrently, metabolomics techniques should be utilized to decipher the *in vivo* pharmacodynamic material basis of the formula, thereby refining the mechanistic framework of the Jiedu Sanjie Formula against gastric cancer and facilitating its transition from fundamental research to clinical practice.

In conclusion, this study elucidates that Jiedu Sanjie Formula intervenes in gastric cancer progression through a multiple-ingredient, multiple-target and multiple-pathway network mode. For subsequent experimental prioritization, we propose two component – target – pathway axes with the greatest research potential: (1) stigmasterol–AKT1–PI3K-Akt, supported by the strongest docking affinity and the central role of AKT1 in gastric cancer survival signaling; and (2) cinobufagin – PIK3R1 – PI3K-Akt/MAPK crosstalk, distinguished by multi-target regulatory effects and the unique formula-specific origin of cinobufagin. Validation of these axes through cellular and biochemical experiments will provide critical evidence for translating the network pharmacology predictions into mechanistic insights, and ultimately facilitate precision clinical application of Jiedu Sanjie Formula in PI3K-Akt–driven gastric cancer subtypes.

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Not applicable.

Conflicts of Interest

The authors declare that there is no conflict of interests.

Author Contributions

Y.F. conceived and designed the study, performed experiments and drafted the manuscript. L.Z. and J.H. collected and analyzed experimental data. R.X. and the corresponding author Y.F. acquired research funding, interpreted results and revised the manuscript. All authors approved the final version of the manuscript.

Ethics Approval and Consent to Participate

No ethical approval was required for this article.

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Availability of Data and Materials

The data presented in this study are available on request from the corresponding author.

Supplementary Material

The following supporting information can be downloaded at: https://ojs.exploverpub.com/index .php/jecacm/article/view/385/sup. Table S1: The specific grid center coordinates for each ligand-target pair.

References

[1] Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians* 2024; 74: 229-263.

[2] Liu P, Kang L, Mang C, et al. Research Progress on the Mechanism of Traditional Chinese Medicine Regulating Immune Evasion to Improve Digestive Tract Tumors. *Chinese Journal of Experimental Traditional Medical Formulae* 2025; 07: 1-12.

[3] Wu W. Alternative and Complementary Effect of Chinese Medicine in Treating Malignant Tumor. *Chinese Journal of Integrated Traditional and Western Medicine* 2011; 31: 111-114.

[4] Cao L, Wang X, Ma X, et al. Exploration of Constitutional Bias and Associated Factors in Patients with Recurrent and Metastatic Gastric Cancer, and the Therapeutic Effect of Yiqi Wenyang Jiedu Prescription: A one-arm study. *Integrative Cancer Therapies* 2025; 24:15347354251331100.

[5] Liu S, Tang L, Zhao Y, et al. Nursing perspective of expert consensus for diagnosis and treatment of gastric cancer with integrated traditional Chinese and Western medicine. *Journal of Integrative Nursing* 2023 5: 8-14.

[6] Li J, Jia J, Zhu W, et al. Therapeutic effects on cancer of the active ingredients in rhizoma paridis. *Frontiers in Pharmacology* 2023; 14: 1095786.

[7] Nanjing University of Chinese Medicine. *Dictionary of Traditional Chinese Medicine*. Shanghai Scientific and Technical Publisher: Shanghai, China, 2006.

[8] Pan J, Wang H, Chen Y. Prunella vulgaris L. - A Review of its Ethnopharmacology, Phytochemistry, Quality Control and Pharmacological Effects. *Frontiers in Pharmacology* 2022; 13: 903171.

[9] Chinese Pharmacopoeia Commission. *Pharmacopoeia of the People's Republic of China (Volume I)*. China Medical Science Press: Beijing, China, 2020.

[10] You L, Geng H, Yang X, et al. The comparison analysis of polyphyllin I and its analogues induced apoptosis of colon and lung cancer cells via mitochondrial dysfunction. *Basic & Clinical Pharmacology & Toxicology* 2021; 129: 15-25.

[11] Tian H, Zhao F, Yue B, et al. Combinational Antitumor Strategies Based on the Active Ingredients of Toad Skin and Toad Venom. *Drug Design, Development and Therapy* 2024; 18: 3549-3594.

[12] Shu T, Liu R, Zhou B. Research Progress on the Chemical Constituents and Bioactivity of the Paris Plants search Progress on the Chemical Constituents and Bioactivity of the Paris Plants. *Shandong Chemical Industry* 2021; 50: 109-130.

[13] Wang Y, Yang J , Yu L. Research progress on chemical ingredients and clinical applications of Bufonis Corium. *Lishizhen Medicine and Materia Medica Research* 2009; 20: 1213-1214.

[14] Fang J, Wang L, Wu T, et al. Network pharmacology-based study on the mechanism of action for herbal medicines in Alzheimer treatment. *Journal of Ethnopharmacology* 2017; 196: 281-292.

[15] Li X, Tang H, Tang Q, et al. Decoding the Mechanism of Huanglian Jiedu Decoction in Treating Pneumonia Based on Network Pharmacology and Molecular Docking. *Frontiers in Cell and Developmental Biology* 2021; 9: 638366.

[16] Jin J, Chen B, Zhan X, et al. Network pharmacology and molecular docking study on the mechanism of colorectal cancer treatment using Xiao-Chai-Hu-Tang. *PLoS One* 2021; 16: e0252508.

[17] Wu Q, Lin J, Liu Y, et al. Study on traditional effect of Panax notoginseng based on network pharmacology. *Chinese Traditional and Herbal Drugs* 2020; 51: 3717-3727.

[18] Wang L, Zhang Y. Mechanism of Qinghua Niwei Decoction in inhibiting "inflammation-cancer" transformation

of CAG with LGIN based on network pharmacology. *Journal of Sichuan of Traditional Chinese Medicine* 2025; 43: 160-168.

[19] Xu L, Yang C, Lu Y, et al. Mechanism of Yishen Qubi Tongluo Formula in the treatment of rheumatoid arthritis based on network pharmacology, molecular docking and experimental validation. *Journal of Traditional Chinese Medicine* 2026; 67: 557-566.

[20] López MJ, Carbajal J, Alfaro AL, et al. Characteristics of gastric cancer around the world. *Critical Reviews in Oncology/Hematology* 2023; 181: 103841.

[21] Chi Z, Zhang H, Cai Y, et al. Quercetin inhibits migration of oxaliplatin-resistant gastric cancer cells through PI3K/AKT signaling pathway. *Practical Pharmacy and Clinical Remedies* 2024; 27: 81-85.

[22] Hang Y, Liao Y, Shen Y, et al. Quercetin enhances 5-fluorouracil-induced apoptosis of gastric cancer cells by inhibiting c-Jun expression. *Chinese Journal of Pathophysiology* 2018; 34: 206-211.

[23] Wei J, Fan Y, Zhang Y, et al. Effects of Quercetin on proliferation of gastric cancer cells and its mechanism. *Shandong Medical Journal* 2007; 35: 14-16.

[24] Song H, Bao J, Wei Y, et al. Kaempferol inhibits gastric cancer tumor growth: An in vitro and in vivo study. *Oncology Reports* 2015; 33: 868-874.

[25] D Y, Feng L, Li Z Y, et al. Efficacy of luteolin on the human gastric cancer cell line MKN45 and underlying mechanism. *Journal of Traditional Chinese Medicine* 2023; 43: 34-41.

[26] Wang J, Liu J, Chen F, et al. The effect of β-sitosterol on cytotoxicity of human costimulatory cells against gastric cancer SGC-7901 cells and its mechanism. *Immunological Journal* 2014; 30: 578-584.

[27] Zhao H, Zhang X, Wang M, et al. Stigmasterol Simultaneously Induces Apoptosis and Protective Autophagy by Inhibiting Akt/mTOR Pathway in Gastric Cancer Cells. *Frontiers in Oncology* 2021; 11: 629008.

[28] Li K, Yuan D, Yan R, et al. Stigmasterol exhibits potent antitumor effects in human gastric cancer cells mediated via inhibition of cell migration, cell cycle arrest, mitochondrial mediated apoptosis and inhibition of JAK/STAT signalling pathway. *Journal of BUON* 2018; 23: 1420-1425.

[29] Wang Y. Cinobufotalin Reverses Cisplatin Resistance in SGC7901 Gastric Cancer Cells via AKT/mTOR Signaling Pathway in vitro. Master's Degree. Harbin Medical University: Harbin, China, 2023.

[30] Zheng Z, Zhang Z, Chen W, et al. Cinobufotalin promotes ferroptosis of gastric cancer cells by regulating the SUMO modification of HIF1 α . *Chinese Pharmacological Bulletin* 2024; 40: 1342-1349.

[31] Ye L, Zheng Z, Chen W, et al. Effects of Cinobufotalin on proliferation and apoptosis of human gastric cancer MKN-28 cells. *The Journal of Medical Theory and Practice* 2024; 37: 908-911.

[32] Zhang A, Zou X, Yang S, et al. Effect of NETs/COX-2 pathway on immune microenvironment and metastasis in gastric cancer. *Frontiers in Immunology* 2023; 14: 1177604.

[33] Li J, Han T. Comprehensive analysis of the oncogenic roles of vascular endothelial growth factors and their receptors in stomach adenocarcinoma. *Heliyon* 2023; 9: e17687.

[34] Morgos D T, Stefani C, Miricescu D, et al. Targeting PI3K/AKT/mTOR and MAPK Signaling Pathways in Gastric Cancer. *International Journal of Molecular Sciences* 2024; 25: 1848.

[35] Chen, L., He, G., Liu, H.. Autophagy-centered regulation of PI3K/Akt/mTOR and MAPK signaling by traditional Chinese medicine in gastric cancer. *Tissue & cell* 2026; 101: 103409.

[36] Thorpe L, Yuzugullu H, Zhao J. PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. *Nature Reviews Cancer* 2015; 15: 7-24.

[37] Hu Q, Dou N, Wu Q, et al. JAK3/STAT5 signaling-triggered upregulation of PIK3CD contributes to gastric carcinoma development. *Journal of Cell Communication and Signaling* 2024; 18: e12017.