Research Paper

Gynostemma pentaphyllum against Gastric Cancer: A Mechanism Study Based on Network Pharmacology, Molecular Docking and *In Vitro* Validation

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***Gynostemma pentaphyllum* is commonly used to treat various tumors in China. The aim of the study is to explore the pharmacological mechanism of *Gynostemma pentaphyllum* in gastric cancer using joint network pharmacology, molecular docking and *in vitro* experimental verification. First, the active constituents and potential targets of *Gynostemma pentaphyllum* were screened from a public database and screening of core targets anti-gastric cancer using protein-protein interaction networks. The Cancer Genome Atlas and Human Protein Atlas databases were used to evaluate the messenger ribonucleic acid and protein expression of core target genes in normal gastric epithelium and gastric cancer tissues and their relationship with overall survival in gastric cancer. Functional and pathway enrichment analyses of the potential targets were performed using gene ontology and Kyoto encyclopedia of genes and genomes. Fifteen active components and all related targets of *Gynostemma pentaphyllum* were retrieved from the Traditional Chinese Medicine Systems Pharmacology database and 127 potential targets were identified by intersection with colorectal cancer-related targets. Protein-protein interaction network analysis showed that six target genes, AKT serine/threonine kinase 1, Jun proto-oncogene and B-cell lymphoma 2 proteins were key genes. Gene ontology enrichment analysis involved 1892 BP, 37 CC and 142 MF. Kyoto encyclopedia of genes and genomes enrichment analysis showed that the anti-cancer effects of *Gynostemma pentaphyllum* were mediated by advanced glycation end products-receptor for advanced glycation end products, interleukin-17, hypoxia inducible factor 1 and transforming growth factor signalling pathways. Molecular docking revealed that the three core target proteins stably bound to quercetin and rhamnazin. The results of the *in vitro* experiments showed that both quercetin and rhamnazin inhibited the activity of gastric cancer cells, up-regulated the messenger ribonucleic acid and protein AKT serine/threonine kinase 1 and down-regulated Jun proto-oncogene and B-cell lymphoma 2 protein activities at the specified concentrations. This study revealed the potential role of *Gynostemma pentaphyllum* in the treatment of gastric using network pharmacology, molecular docking and *in vitro* experiments.**

Key words: *Gynostemma pentaphyllum*, gastric cancer, network pharmacology, molecular docking, experimental validation

Gastric Cancer (GC) remains a formidable adversary in the global battle against malignant tumors. It is consistently ranked as the fourth most common and second most lethal cancer worldwide[1]. The disparity between the high curability of early stage GC and the grim prognosis of advanced stages is stark with the latter exhibiting a high propensity for metastasis, significant mortality, diminished likelihood of effective surgical removal and a dismal 5 y survival rate[2]. The prognosis for most patients is poor, predominantly because of the challenges of distant metastasis and recurrent disease[3]. While advancements in early detection and surgical

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interventions have markedly enhanced the treatment outcomes for GC, the 5 y survival rate remains below 30 %[4]. Chemotherapy, another cornerstone of GC management is associated with considerable adverse effects and a high risk of developing drug resistance[5]. Natural anticancer compounds are emerging as a beacon of hope because of their superior efficacy, minimal side effects and low toxicity[6], making the exploration of these agents an invaluable endeavour for enhancing cancer treatment and patient outcomes.

Gynostemma pentaphyllum (GPH), a member of the Cucurbitaceae family, is predominantly found in Northeast and Southeast Asia[7]. Previous studies have highlighted its multifaceted effects, including anti-inflammatory properties[8], antioxidant stress resistance[9], immunomodulation[10], anti-aging benefits[11] and cardiovascular disease prevention[12] with its anticancer activity being particularly notable[13]. GPH contains various chemical substances such as saponins, polysaccharides and flavonoids[7]. Both saponins and flavonoids have been reported to possess antiproliferative effects against a wide range of tumor cell lines, including liver cancer[14], colorectal cancer[15], glioma[16], oral cancer[17], tongue cancer[18] and lung cancer[19]. Network pharmacology is a novel and valuable approach that leverages systematic network models to analyze the interactions among Chinese medicine formulas, diseases, targets and pathways[20], providing a theoretical foundation for further research on natural medicines[21]. Accordingly, we employed network pharmacology to identify the potential targets of GPH and unveil its complex mechanisms in the treatment of GC. Furthermore, we validated the molecular docking results through *in vitro* cell experiments, offering a reliable theoretical basis for the application of GPH in GC treatment. The detailed study process is shown in fig. 1.

MATERIALS AND METHODS

Network pharmacological analysis:

Screening of effective components and targets of GPH: The main components of GPH were extracted from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database[22]. Active ingredients were screened based on oral bioavailability ≥30 % and drug similarity ≥0.18 thresholds and their corresponding targets were collected. Uniprot was used to convert targets to standardized gene names and select validated protein data.

Identification of GC-related gene targets: The targets related to GC were retrieved from the human Online Mendelian Inheritance in Man (OMIM) database (https://omim.org/)[23] and GeneCards (https://www.genecards.org/)[24], utilizing "gastric cancer" as the search keyword. The redundant target genes were removed. Venn diagram depicting the intersection of genes was created to identify the potential targets of GPH in combating gastric cancer.

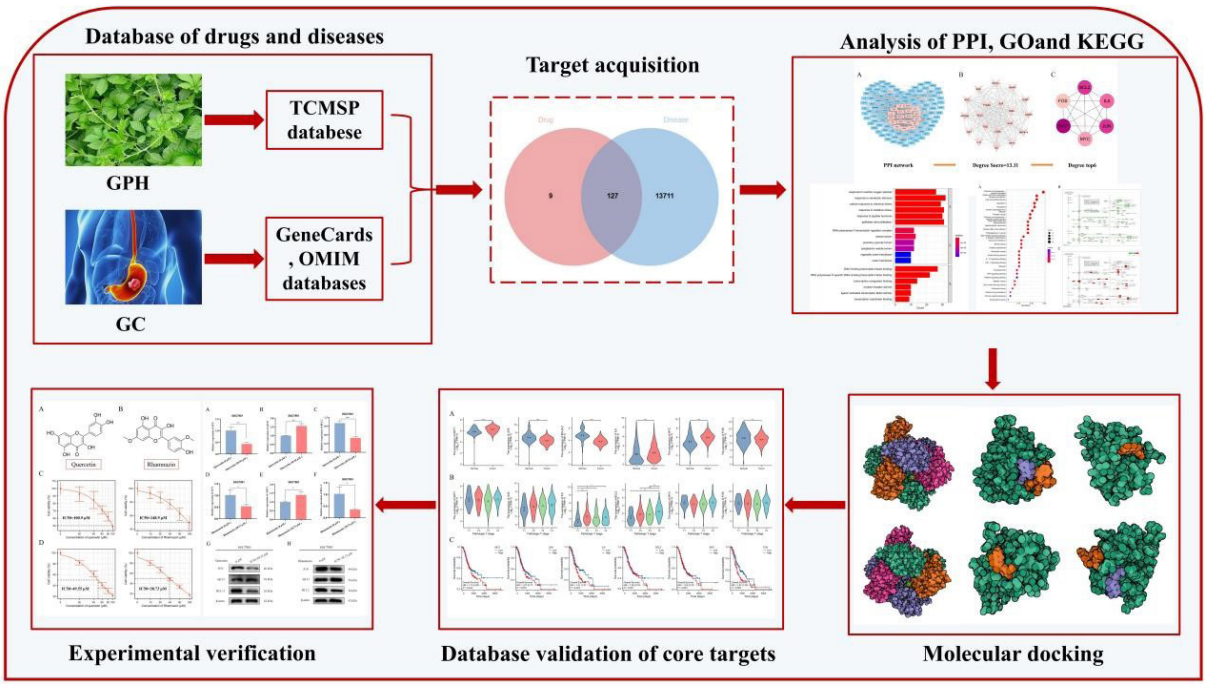

Fig. 1: Research flow chart

Drug-ingredient-target-disease network construction: The active ingredients and targets of GPH were integrated into Cytoscape 3.8.2 to construct the network diagram illustrating the "GPH-active ingredients-target-GC" interactions. In this network diagram, the nodes symbolize the action targets, active components, GC and GPH whereas the edges denote the interactions between them.

Construction of Protein-Protein Interaction (PPI) networks: The intersecting targets of GPH and GC were entered into the STRING database (https://cn.string-db.org/cgi/input.pl), selecting "*Homo sapiens*" as the species, with the interaction confidence threshold set to medium confidence ≥0.7. Disconnected nodes were hidden to generate a PPI network diagram. The results were then imported into Cytoscape 3.8.2 for network construction and topological analysis and the key targets within the network were filtered. The selection parameters for the core targets included Degree Centrality (DC).

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis: Enrichment analysis was conducted using the Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/)[25] and the "Disease Target Network". GO enrichment for graphene oxide included Biological Processes (BP), Cellular Components (CC) and Molecular Functions (MF). For GO and KEGG, an enrichment $p<0.05$ was considered statistically significant. The disease-target pathway network was visualized using Cytoscape (version 3.8.2).

Validation of key targets in The Cancer Genome Atlas (TCGA)-Genotype-Tissue Expression (GTEX) and Human Protein Atlas (HPA) databases:

To validate the key targets against TCGA (https://portal.gdc.cancer.gov)[26], we downloaded and organized Ribonucleic Acid (RNA)-sequence data processed from the TCGA GTEX and TCGA STAD projects. The mRNA expression of six key genes in TPM format was extracted to assess the Overall Survival (OS) of patients with GC with high or low expression of these key genes. HPA (https://www.proteinatlas.org/)[27] was used to retrieve the expression and distribution of proteins in the normal stomach and gastric tissues for these six key targets.

Molecular docking:

Core active components and targets of GPH were selected for molecular docking. The two-dimensional structures of the core active components were downloaded from the TCMSP database and set in mol2 format. Three-dimensional structures of the target proteins were downloaded from the Protein Data Bank (PDB, https://www.rcsb.org/)[28] and saved in the PDB format. PyMOL software[29] was used to remove water molecules and small-molecule ligands from the target protein structures. AutoDockTools-1.5.6[30] was used to add hydrogen and charge to the active components and target proteins to construct docking pockets for molecular docking. PyMOL software was used to visualize the docking results.

Validation of *in vitro* experiments:

Cell culture, drug source and viability analysis: SGC7901 GC cells were cultured in Roswell Park Memorial Institute-1640 medium supplemented with 10 % Fetal Bovine Serum (FBS) and 1 % penicillin-streptomycin in a humidified incubator at 37° and 5 % CO_2. Quercetin (No.: HY-18085) and rhamnazin (Case No.: HY-N8342) were acquired from MedChemExpress (https://www.medchemexpress.cn/), dissolved in Dimethyl Sulfoxide (DMSO) and diluted in the medium to sequential concentrations (final DMSO concentration of 0.1 %). To assess the inhibitory effects of quercetin and rhamnetin on cell viability, logarithmically growing SGC7901 cells were seeded in 96-well plates at a density of 5×10^4 cells per well and treated with the specified concentrations of quercetin, rhamnetin or vehicle (medium containing 0.1 % DMSO) for 48 h. After treatment, 10 µl of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) solution was added to each well and incubated at 37° for 30 min. The absorbance was measured at 450 nm using a Bio-Rad spectrophotometer (USA).

Real Time-qualitative Polymerase Chain Reaction (RT-qPCR) analysis: Total RNA was extracted from SGC7901 cells treated with different concentrations of quercetin and rhamnazin for 48 h using a total RNA rapid extraction kit. The total RNA concentration was determined using a NanoDrop one spectrophotometer (Thermo Scientific, USA). Total RNA was reverse-transcribed to complementary Deoxyribonucleic Acid (cDNA) using the HiFiScript genomic Deoxyribonucleic Acid (gDNA) removal cDNA synthesis kit. The Polymerase Chain Reaction (PCR) system was prepared using the SYBR Green

PCR master mix (Low ROX) kit, cDNA was amplified and a Roche LightCycler 480 (Roche Diagnostics, Basel, Switzerland) was used. The relative expression of target genes was calculated by the $2^{-\Delta\Delta Ct}$ method. The PCR primer sequences are listed in Table 1.

Western blot: SGC7901 GC cells (1×10^6) were seeded into 100 cm diameter culture dishes. Fresh culture medium containing quercetin (49.55 μm) and rhamnetin (38.73 μm) was added to the dishes and incubated for 48 h. Cells were collected by washing with cold Phosphate-Buffered Saline (PBS) and scraping. Cells were lysed on ice using lysis buffer (Shanghai, China). The protein concentration was determined using a Bromocresol Green Albumin (BCA) protein assay kit (KeyGEN BioTECH, Jiangsu, China). Equal amounts of protein were loaded onto 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels and subsequently transferred onto polyvinylidene fluoride membranes. The membranes were blocked with 5 % non-fat milk for 1 h and incubated overnight at 4° with primary antibodies. After washing thrice with Tris-Buffered Saline, 0.1 % Tween 20 (TBS-T) solution (10 min each time), the membranes were incubated with secondary antibody (1:2000) for 1 h. After three washes with TBS-T (10 min each), the blots were visualized using an Enhanced Chemiluminescence (ECL) kit (GE Healthcare Life Sciences, Marlborough, MA, USA). Images were captured using a ChemiDoc XRS+Imaging System (Bio-Rad, Hercules, CA, USA). Anti-AKT serine/threonine kinase 1 (AKT1) (80816-1-RR, 1:10000), anti-Jun proto-oncogene (JUN) (222114-1-AP, 1:600) and anti-B-Cell Lymphoma 2 (BCL2) (68103-1-Ig, 1:10000) antibodies were purchased from Proteintech (Wuhan, China). Horseradish peroxidase-conjugated secondary antibody (Thermo Fisher Scientific, Shanghai, China) was used for each protein sample with the band intensity normalized to their own internal standard protein (β-actin).

Statistical analysis:

All data parts of the network pharmacology analysis were automated using software or databases, such as the p-values for GO and KEGG, which were statistically analyzed automatically using the DAVID database. All experimental data are presented as mean±Standard Deviation (SD). Univariate analysis of variance was performed using Prism 9.0. Differences were considered statistically significant at *$p<0.05$, **$p<0.01$ and **$p<0.001$.

RESULTS AND DISCUSSION

The active components of GPH derived from Traditional Chinese Medicine (TCM), were screened meticulously. After eliminating duplicates, 15 active ingredients and 136 target genes that fulfilled the selection criteria were identified. The active pharmaceutical ingredients are listed in Table 2.

GC-related targets were identified through searches of the GeneCards and OMIM databases, yielding 13 838 and 168 unique targets respectively, after duplicates were removed. Venn software facilitated the amalgamation of targets from both GeneCards and OMIM culminating in a comprehensive list of 13 838 GC-related targets. By analyzing the overlap between these GC-related targets and the targets of GPH's active ingredients, 127 potential targets for therapeutic intervention in GC were identified, as illustrated in fig. 2.

TABLE 1: CLINICAL INFORMATION OF THE TWO GROUPS

Genes	Sequence (5'-3')
AKT1	F: ACTGTCATCGAACGCACCTT
	R: CTCCTCCTCCTCCTGCTTCT
JUN	F: GTGCCGAAAAAGGAAGCTGG
	R: GCTGCGTTAGCATGAGTTGG
BCL2	F: GGTGAACTGGGGGAGGATTG
	R: ATCCCAGCCTCCGTTATCCT
GAPDH	F: GGAGCGAGATCCCTCCAAAAT
	R: GGCTGTTGTCATACTTCTCATGG

TABLE 2: ACTIVE PHARMACEUTICAL INGREDIENTS OF GPH

MOL ID	Molecule name	OB (%)	DL
MOL000338	3'-methyleriodictyol	51.61	0.3
MOL000351	Rhamnazin	47.14	0.3
MOL000359	Sitosterol	36.91	0.8
MOL004350	Ruvoside_qt	36.12	0.8
MOL004355	Spinasterol	42.98	0.8
MOL005438	campesterol	37.58	0.7
MOL005440	Isofucosterol	43.78	0.8
MOL000953	CLR	37.87	0.7
MOL000098	Quercetin	46.43	0.3
MOL009855	(24S)-ethylcholesta-5,22,25-trans-3beta-ol	46.91	0.8
MOL009867	4α,14α-dimethyl-5α-ergosta-7,9(11),24(28)-trien-3β-ol	46.29	0.8
MOL009877	Cucurbita-5,24-dienol	44.02	0.7
MOL009878	Cyclobuxine	84.48	0.7
MOL009971	Gypenoside XXVII_qt	30.21	0.7
MOL009973	Gypenoside XXVIII_qt	32.08	0.7

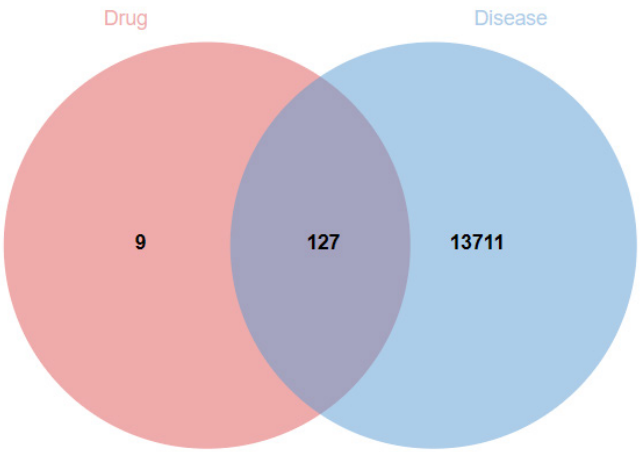

Fig. 2: Venn diagram of the targets of GPH and GC

The network diagram illustrating the "GPH-active ingredient-target-GC" interactions for GPH's therapeutic approach to GC was constructed utilizing Cytoscape 3.8.2, as depicted in fig. 3. This diagram features 144 nodes and 312 edges, highlighting GPH's multifaceted and targeted strategy of the GPH to combat GC.

The shared targets of GPH and GC were examined using the STRING database to assemble a PPI network. After downloading the data and PPI network diagram, where nodes symbolized proteins and edges delineated the interactions among them, the findings were imported into Cytoscape 3.8.2. This facilitated the construction of the network and its topological analysis, thereby identifying critical targets within the network. Using DC as the selection criterion, AKT1, JUN, BCL2, IL6, Myelocytomatosis oncogene (MYC) and Fos proto-oncogene (FOS) were identified as pivotal targets of GPH in GC treatment (fig. 4A-fig. 4C).

Utilizing the DAVID database, a comprehensive analysis yielded 2027 processes with 1892 pertaining to BP. The identified targets were predominantly engaged in responses to oxidative stress, epithelial cell proliferation and cellular responses to chemical stress. Concerning CC, 37 entries were noted,

focusing mainly on aspects such as the RNA polymerase II transcription regulator complex and vesicle lumen. For MF, 142 entries were identified, primarily involving interactions with DNA-binding transcription factors, RNA polymerase II-specific DNA-binding transcription factor binding, transcription co-regulator binding, nuclear receptor activity, ligand-activated transcription factor activity and transcription co-activator binding. The top six entries from the BP, CC and MF were selected for depiction, as shown in fig. 5A. Moreover, 170 signalling pathways were identified that were primarily associated with cancer, viral infections and inflammation-related pathways, including those related to GC, breast cancer, Epstein-Barr virus infection, Advanced Glycation End products-Receptor for Advanced Glycation End products (AGE-RAGE) signalling, Interleukin (IL)-17 signalling and Hypoxia-Inducible Factor 1 (HIF-1) signalling. The top 30 pathways are presented as bubble charts in fig. 5B. In addition, we showed the distribution of GC pathways (fig. 6A and fig. 6B).

Analysis of TCGA database indicated that the mRNA levels of AKT1, IL6 and MYC were significantly higher in GC tissues, with JUN, BCL2 and FOS being expressed at low levels (fig. 7A). Furthermore, we explored the association between mRNA levels of key targets, the pathological stage and OS in GC. The findings showed that IL-6 and BCL2 levels varied with the disease stage (fig. 7B). Elevated levels of IL-6 and FOS were associated with worse outcomes in patients with GC ($p<0.05$; fig. 7C). Additionally, data from the HPA database demonstrated the differential expression of major target proteins in normal stomach tissues. In GC tissues, the protein expression levels of AKT1 and MYC were higher than those in normal gastric tissues, whereas JUN and FOS showed reduced expression levels (fig. 8).

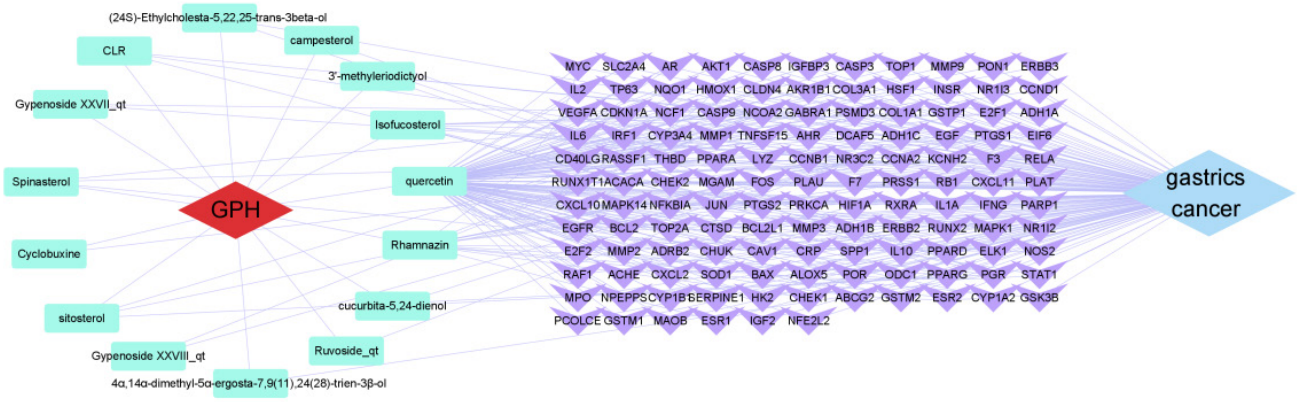

Fig. 3: Network construction of GPH active components-GC targets

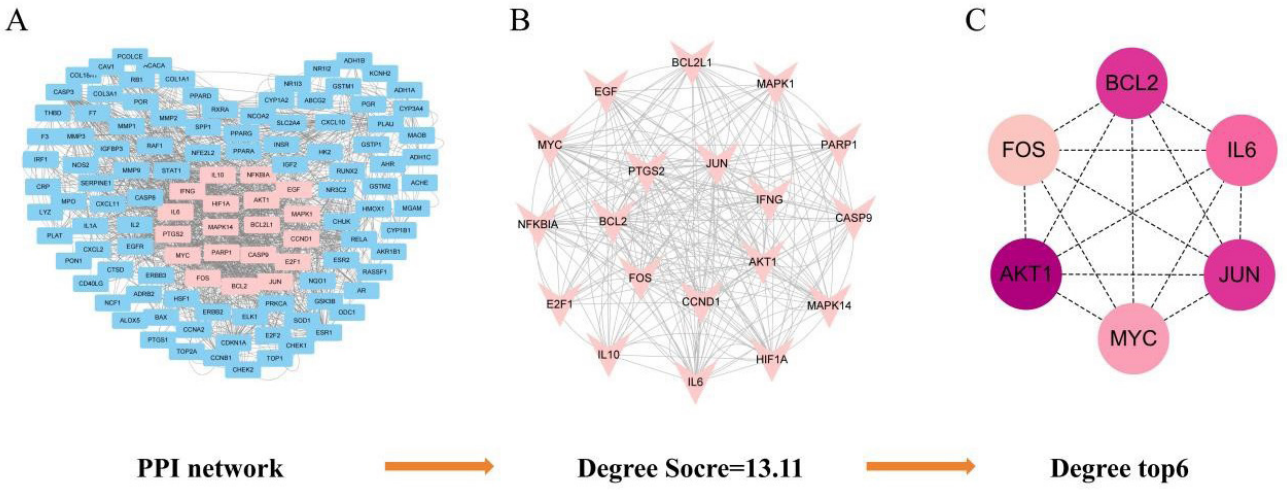

Fig. 4: Analysis of the target protein–protein interaction network in GPH treatment of GC

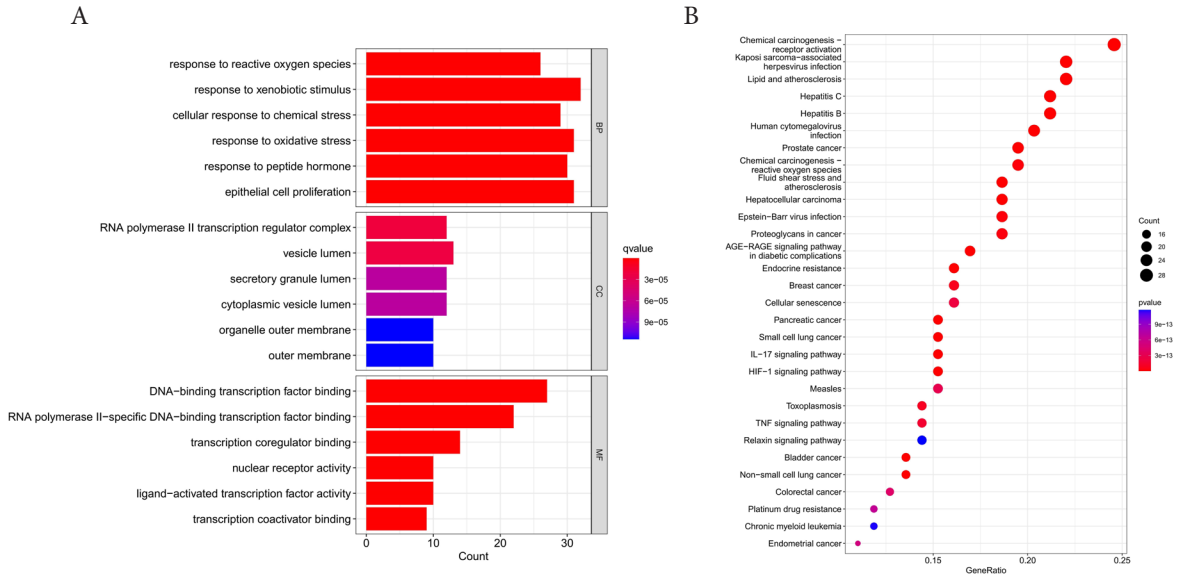

Fig. 5: GO and KEGG enrichment analysis of targets in the treatment of GC using GPH

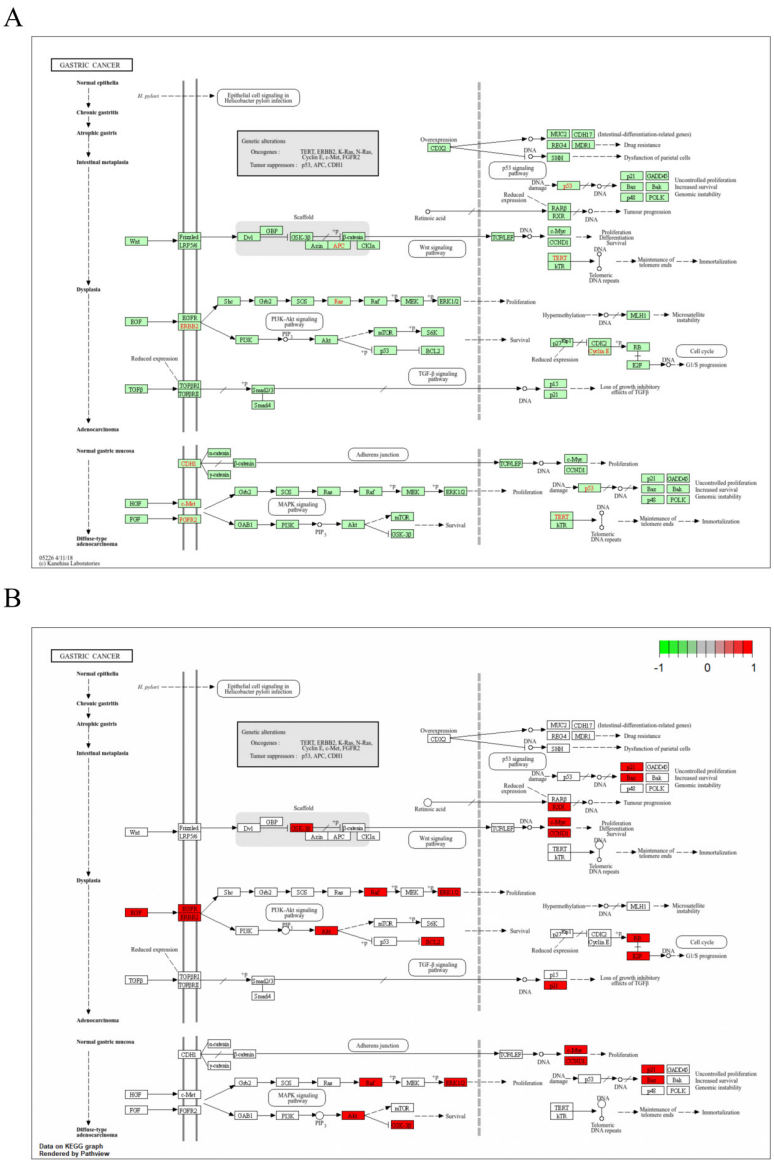

Fig. 6: Pathways in gastric cancer

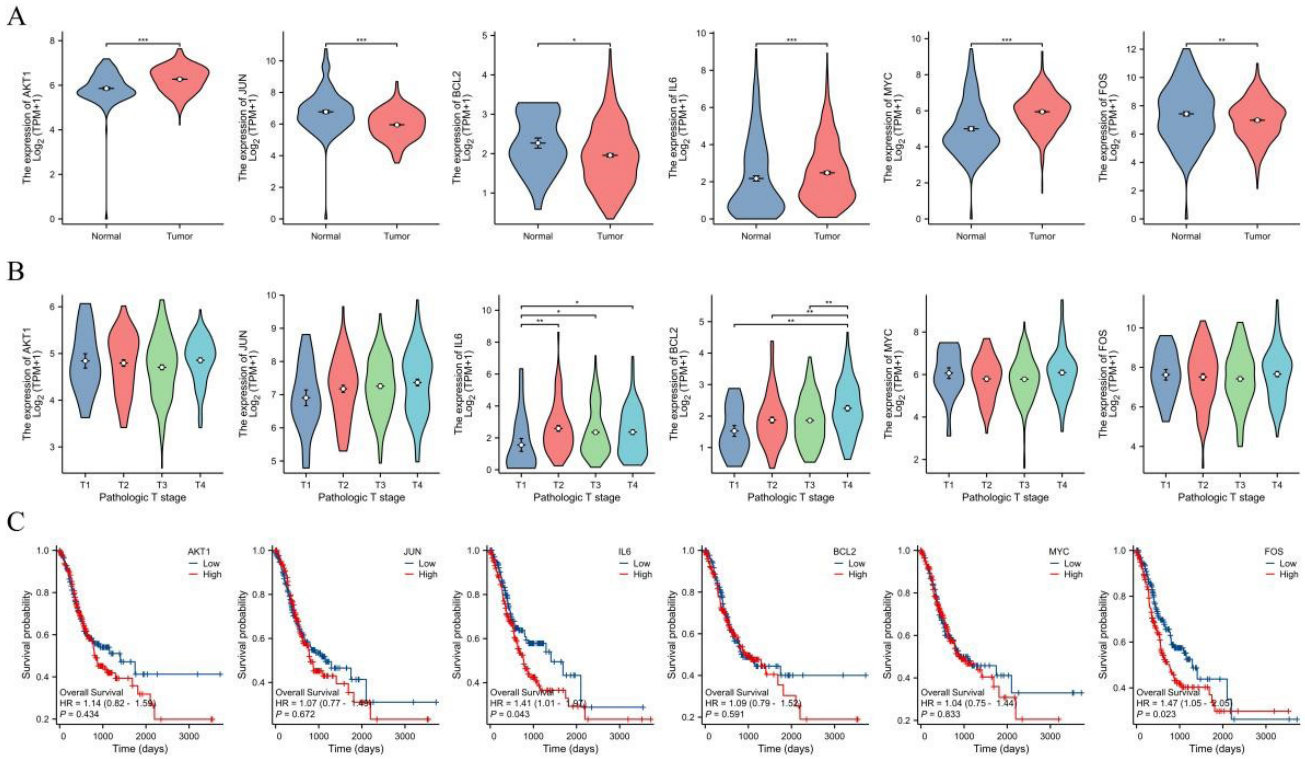

Fig. 7: mRNA expression and overall survival of key genes in the TCGA database

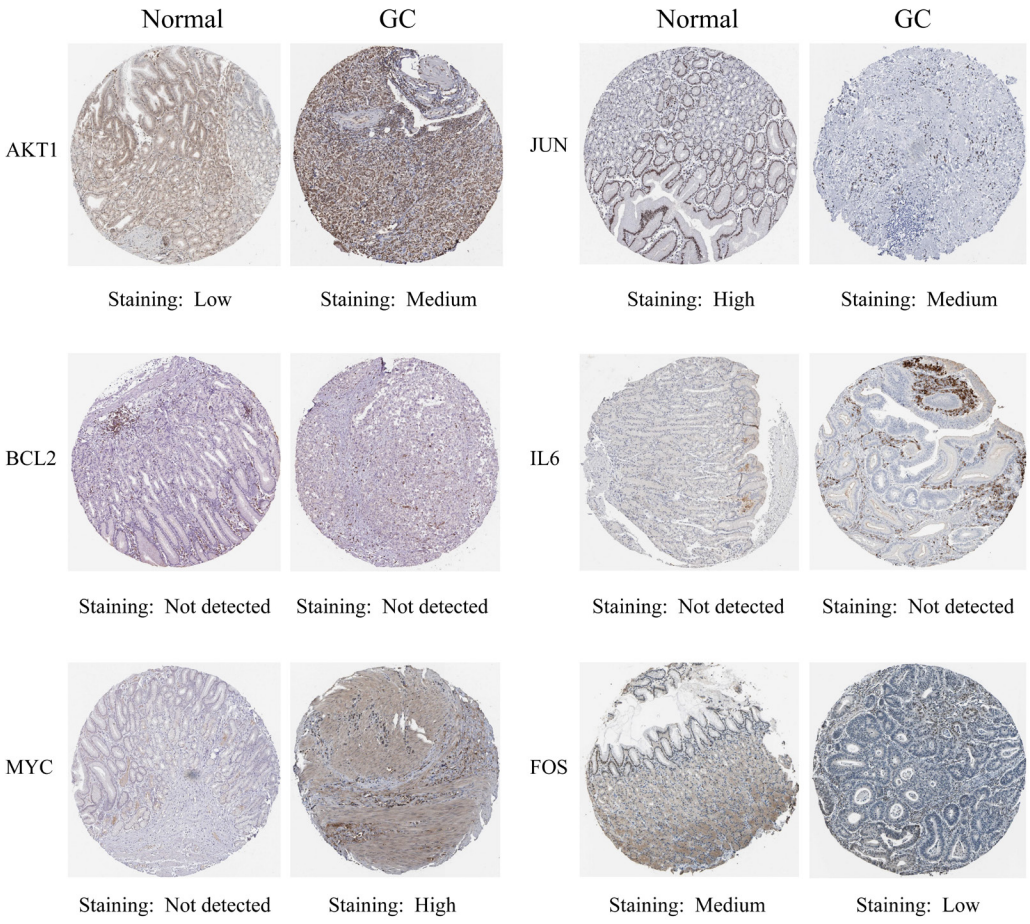

Fig. 8: Analysis of protein expression levels of key targets in HPA databases

Molecular docking techniques were used to evaluate the binding affinities of quercetin and rhamnetin, the principal active compounds of GPH to their central targets. Using PyMOL software, the binding orientation and interactions between quercetin and rhamnetin with key targets, such as AKT1, JUN and BCL2, were meticulously analyzed and the binding energies for each interaction were calculated. Lower binding energies indicate enhanced the stability of the ligand-receptor complex. The docking results were visualized using PyMOL software, culminating in a detailed molecular docking model depicting the interactions (fig. 9).

First, we acquired the molecular structures of quercetin and rhamnazin from MCE's official website (fig. 10A and fig. 10B). We then used the MTS test to investigate the effects of various drug doses on the activity of GC cells. The findings revealed that quercetin and rhamnazin inhibited the activity of GC cell line SGC7901 in a concentration-dependent manner, with a median Inhibitory Concentration (IC_{50}) of 49.55 µm and 38.73 µm, respectively (fig. 10C and fig. 10D).

The effects of quercetin and rhamnetin on the mRNA expression of AKT1, JUN and BCL2 in SGC7901 cells were assessed using RT-PCR. Relative to the control group, the mRNA expression of AKT1, JUN and BCL2 was significantly reduced after 48 h of exposure to the designated concentrations of quercetin and rhamnetin (fig. 11A-fig. 11F). To corroborate the RT-PCR results, Western blotting was performed to examine the protein expression levels of AKT1, JUN and BCL2. Fig. 11G and fig. 11H illustrate that, compared to the control group, treatment with quercetin and rhamnetin for 48 h in SGC7901 cells resulted in an upregulation of AKT1 protein expression, whereas the expression levels of JUN and BCL2 proteins were markedly decreased.

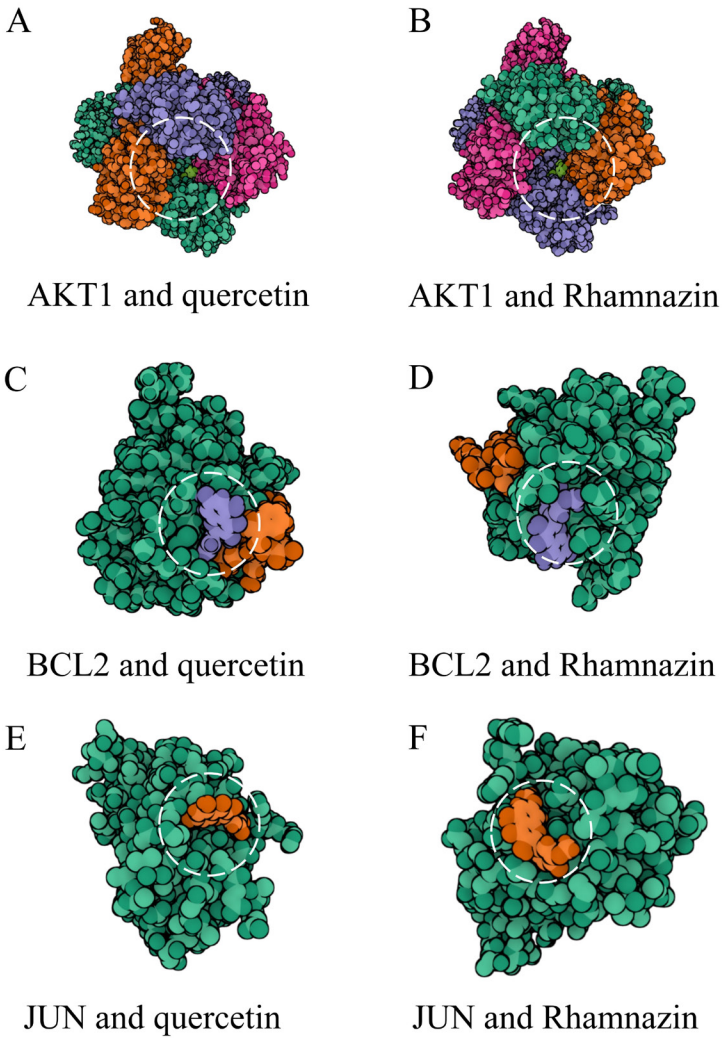

Fig. 9: Molecular docking of the active components with key targets

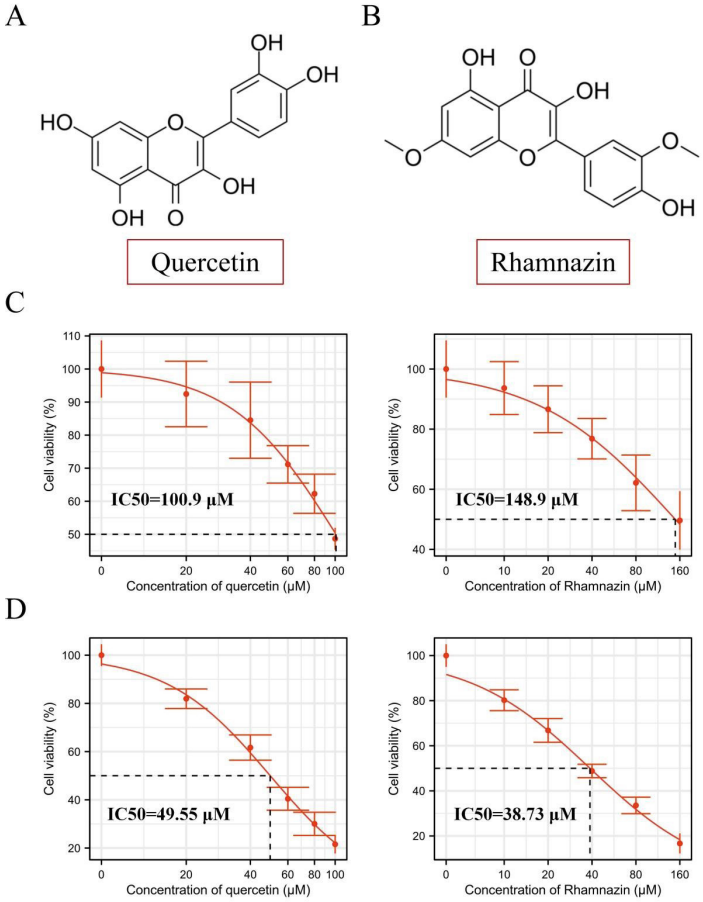

Fig. 10: The effects of quercetin and rhamnetin on SGC7901 cells and normal cell activity

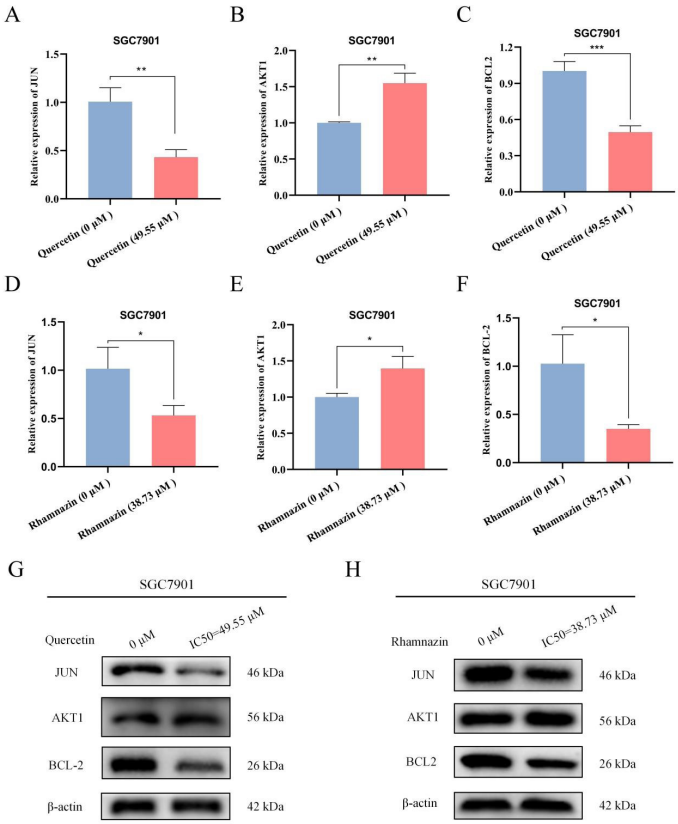

Fig. 11: Effects of quercetin and rhamnazin on mRNA and protein expressions of AKT1, JUN and BCL2 in GC cells

In recent years, the incidence and mortality of GC have gradually increased and are related to diet, lifestyle, genetics and other factors. Most patients with GC have a poor prognosis owing to the possibility of distant metastasis and recurrence[3]. In the present study, we used a comprehensive approach combining network pharmacology and molecular docking to investigate the role of GPH in the treatment of GC. This study is the first to reveal the inhibitory effect of core active ingredients, such as quercetin and rhamnazin, on the activity of GC cells and that the regulation of core targets may reveal its anticancer mechanism. This finding provides experimental validation of its potential application in the treatment of GC. The pharmacological effects and complex mechanisms of TCM, characterized by its multi-component, multi-target and multi-pathway interactions have become a focal point in the research on Chinese medicine for cancer treatment[31].

We utilized network pharmacology to compile the active components of GPH and their targets, as well as the disease targets of GC, from relevant databases. GPH shares 127 common targets with GC, of which quercetin and rhamnetin have been identified as the principal core components. Research has found that flavonoids, phytosterols and saponins, including quercetin and rhamnetin, are the active components of GPH used in the treatment of coronary heart disease[32]. Additionally, studies have explored the mechanisms by which GPH treats atherosclerosis using network pharmacology[33]. These results indicate that quercetin is one of the most effective targets of the many active components of GPH, which is consistent with the findings of this study. Furthermore, the PPI network analysis in our study highlighted AKT1, JUN, BCL2, IL-6, MYC and FOS as key targets, demonstrating that their highly efficient interactions are primarily involved in inflammatory responses and cancer pathways. AKT1 is a serine/threonine-specific protein kinase that is pivotal in a wide array of BP, including glucose metabolism, cellular proliferation, apoptosis and migration[34,35]. As an enzymatically active kinase, AKT1 is instrumental in driving the advancement of GC and facilitating the proliferation of GC cells, thus underscoring its significance in cancer progression[36]. The oncogene JUN, a component of the AP-1 transcription factor complex, plays a pivotal role in regulating cell cycle progression, apoptosis and cell differentiation[37]. BCL2 is an oncogene whose expression increases with the grade of GC. Consequently, it enhanced cell proliferation and inhibited programmed cell death[38]. IL-6 is highly upregulated in many cancers, making it one of the most crucial cytokines in the oncogenesis and metastasis of tumors[39]. Moreover, high IL-6 expression serves as a biomarker for poor prognosis[40]. Additionally, it can effectively control pathological changes in cancer by mediating the expression of IL-6 in the IL-17 signaling pathway[41]. We found that high expression of IL-6 was associated with significantly lower survival rates, according to the TCGA database.

GO enrichment analysis indicated that GPH treatment of GC is involved in multiple BP, including responses to oxidative stress, epithelial cell proliferation and cellular responses to chemical stress. KEGG pathway enrichment analysis suggested that the mechanisms by which GPH treats GC may involve key pathways, such as GC, breast cancer, Epstein-Barr virus infection, AGE-RAGE signalling, IL-17 signalling and HIF-1 signalling pathways. During tumorigenesis, reprogramming of cell death mechanisms through AGE-RAGE signal transduction is intriguing for understanding the complex signal transduction mechanisms in cancer cells[42]. The IL-17 cytokine family and its receptors are critical for immune responses and the dysregulation of their expression is associated with inflammation and cancer[43]. The HIF signalling pathway accelerates GC progression by regulating several genes that affect GC cell proliferation, metastasis, apoptosis and angiogenesis. Notably, HIF-1α enhances GC invasion and metastasis by inducing epithelial-mesenchymal transition. HIF-1α is not only a potential mediator in the development of GC but also correlates with the severity of GC and poor prognosis[44,45].

Further molecular docking and analysis were conducted on candidate targets with higher degree values in the core PPI network, namely, AKT1, JUN and BCL2. The results demonstrated that these three core targets exhibit good binding stability to quercetin and rhamnetin. These results are similar to the findings of studies conducted by Ning *et al*.[45], Liu *et al*.[46] and Wang *et al*.[47]. Additionally, *in vitro* experiments demonstrated the inhibitory effects of quercetin and rhamnetin on GC cell activity and validated target regulation by molecular docking, further confirming the stable binding of quercetin and rhamnetin to the targets AKT1, JUN and BCL2. These findings may reveal part of the mechanism by

which GPH treats GC; however, the specific complex mechanisms require further investigation.

However, this study has some limitations. First, our approach for identifying therapeutic targets relies on network pharmacology and molecular docking, which depend on existing databases and the known structures of target proteins. Therefore, our research was limited by the accuracy and timeliness of these databases, as well as the comprehensiveness of compound inclusion. Second, in this study, quercetin and rhamnetin were identified as the most important active components in treating GC. However, the synergistic effects of other active components in GPH on the treatment of GC remain to be explored, providing a direction for future work.

In summary, our study demonstrated that GPH, particularly its major active compounds, quercetin and rhamnazin, may exert therapeutic effects on GC by modulating multiple targets, such as AKT1, JUN and BCL2. Additionally, this study supports the notion that network pharmacology predictions validated by molecular docking can offer a preliminary and systematic exploration of the pharmacokinetic characteristics and mechanisms of TCM in human diseases. Furthermore, by experimentally validating the reliability of the molecular docking techniques, our study provides further opportunities for targeted and precise cancer treatment using TCM.

Author's contributions:

Qincai Li designed and wrote the manuscript. Zhang and Zheng contributed reagents, materials and analysis tools and analyzed the data. HaiYun-Liu performed the experiments. Xiaogang Xu provided critical revisions of the manuscript.

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Data availability statement:

All authors agree to publish the data in this study.

Conflict of interests:

The authors declared no conflict of interests.

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