doi.org/10.36721/PJPS.2026.39.10.273.1

Quercetin as the core constituent of Huanglian decoction: Targeting AKT1 for colorectal cancer therapy

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Abstract: **Background**: Colorectal cancer (CRC) is a prevalent gastrointestinal carcinoma. Huanglian decoction (HD) has been applied to treat gastrointestinal disorders for millennia. **Objectives**: This study utilized network pharmacology strategies to establish the drug–compound–disease target network. **Methods**: To explore the biological functions and pathways involved, GO and KEGG enrichment analyses were conducted. To identify core targets from the findings, a protein-protein interaction (PPI) network was constructed. We utilized the molecular docking assay, CETSA and DARTS to validate the compound-target complex. The biological effects of the key compound on CRC cells were studied using CCK-8 assay, colony-formation assay, scratch test and trans well invasion assay. **Results**: Our findings indicated that half of the HD potential targets were CRC-related genes. PPI analysis identified 7 key genes: AKT1, ESR1, JUN, IL6, MYC, FOS and CCND1. Quercetin was identified as a core active compound of HD, which likely exerts its effects by targeting AKT1 and inhibiting the AKT-mTOR pathway, thereby suppressing the proliferation, migration and invasion of CRC cell lines (Caco-2 and SW480). **Conclusion**: The results suggest that HD has potential therapeutic properties against CRC. Its active ingredient, quercetin, exerted anti-CRC effects by binding to AKT1 and inhibiting the activation of the AKT-mTOR pathway.

Keywords: Colorectal cancer; Huanglian decoction; Molecular docking; Network pharmacology; Quercetin

Submitted on 09-08-2025 – Revised on 04-11-2025 – Accepted on 07-11-2025

INTRODUCTION

The high morbidity of colorectal cancer (CRC) aggravates the global health burden (Morgan *et al*., 2023). Driven by modern dietary habits and environmental pollution, the incidence of early-onset colorectal cancer is surging; epidemiologists presume that about one-third of CRC will occur before the commonly recommended screening age of 50 years by 2030 (Mauri *et al*., 2025). Quite a few patients were diagnosed with an advanced stage (III-IV) of disease, missing the optimal window for surgical intervention (Carbone *et al*., 2025). While chemotherapeutic agents, targeted therapies and immunotherapies constitute pivotal anti-neoplastic strategies, their intended therapeutic outcomes are largely undermined by treatment-associated adverse effects and acquired drug resistance (Ward *et al*., 2003, Orhan *et al*., 2025). Thus, developing novel therapies for CRC is a critical unmet need.

Huanglian decoction (HD) is a traditional Chinese medicine formula first documented in "Sheng Ji Zong Lu" during the Song dynasty (Xiong *et al*., 2022). It contains four traditional Chinese medicinal components: *Coptis chinensis*, dried ginger, dark plum and argyi. HD is known for its "heat-clearing and detoxifying" properties and has a long history of clinical use in treating intestinal diseases, such as ulcerative colitis and Crohn's disease, which are well-established risk factors for CRC (Hirsch *et al*., 2021, Nadeem *et al*., 2020). Therefore, HD may have therapeutic potential against CRC. However, the complex composition of Traditional Chinese Medicine (TCM) formulas presents a challenge for elucidating their mechanisms. Network pharmacology applies computational algorithms to construct disease-drug networks, which were used to systematically reveal bioactive compounds, therapeutic targets and pharmacodynamic mechanisms underlying TCM efficacy (Hopkins, 2007; Nogales *et al*., 2022).

Therefore, we used network pharmacology strategies to identify the critical targets of HD against CRC in this study. Twenty-one active compounds and 102 active compound–disease interaction targets were screened from the drug–compound–disease target network. Subsequently, molecular docking analysis identified quercetin as the potential core constituent, with AKT1 emerging as its most stable binding target protein. Finally, the predicted interaction between quercetin and AKT1, along with quercetin's biological functions in CRC cells, was validated through a series of *in-vitro* experiments.

MATERIALS AND METHODS

Disease-related genes acquisition

CRC-related gene sets were achieved from the Therapeutic Target database (https://db.idrblab.net/ttd/) (Zhou *et al*., 2024), OMIM database (https://www.omim.org) (McKusick, 2007), DrugBank database (https://go.drugbank.com/) (Wishart *et al*., 2006),

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GeneCards database (https://www.genecards.org/) (Stelzer *et al*., 2016) and PharmGKB database (https://www.pharmgkb.org/) (Whirl-Carrillo *et al*., 2021).

Construction of compound-target network

Using the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (https://www.91tcmsp.com/#/) (Ru *et al*., 2014), we screened HD compounds for oral bioavailability ≥ 30% and drug-likeness ≥ 0.18 to identify active components and their targets. Potential therapeutic targets were derived from the overlap between these targets and disease-related genes and a network was constructed in Cytoscape software 3.10.3.

Construction and analysis of protein-protein interaction (PPI) network

The PPI of HD was built using the STRING database (https://string-db.org/). Potential target proteins were imported with the organism set to *Homo sapiens* and a minimum interaction confidence score of 0.4. Then, the resulting network was analyzed in Cytoscape using the cytoNCA plugin to calculate key topological parameters, including betweenness, closeness, degree, eigenvector, LAC and network centrality. Proteins with all parameters higher than the median were considered core targets.

Function enrichment analysis

By uploading potential HD interaction targets into the "clusterProfiler 4.14.4" package, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted. Then, the results, ranked by ascending gene ratio, were presented via the "ggplot2 3.5.1" package.

Molecular docking

The PubChem database was used to obtain the 3D structure of quercetin (https://pubchem.ncbi.nlm.nih.gov/). We downloaded the crystal structures of targets from the PDB database (https://www.rcsb.org/). Following this, both the ligand (quercetin) and the target proteins were prepared by conversion to the PDBQT format. Molecular docking was performed using AutoDock Vina (v1.1.2) and visualized with PyMOL (v3.0.3).

Cell culture

The human CRC cell lines (Caco2 and SW480), purchased from ATCC and confirmed by STR profiling, were cultured in DMEM supplemented with 1% penicillin-streptomycin and 10% FBS (SW480) or 20% FBS (Caco2) at 37°C and 5% CO_2.

Cellular thermal shift assay (CESTA)

The binding of a drug to its target protein can enhance the thermal stability of the protein. (Jafari *et al*., 2014). After being treated with quercetin (20 μmol, MCE, USA) or DMSO for 2 h, SW480 cells were digested with trypsin. Next, cell suspension was collected into the PCR tubes (100 μl/tube), incubated at specific temperatures (44°C, 47°C, 50°C and 53°C) for 3 min. AKT protein level was than measured by Western blot (WB).

Drug affinity responsive target stability (DARTS)

Drug binding to its target protein can reduce the protease susceptibility of the target (Lomenick *et al*., 2009). SW480 cells were digested with trypsin and collected into the PCR tubes (100 μl/tube). Next, cell lysis buffer was used to extract cellular proteins. Treated with quercetin (20 μmol, 40 μmol and 80 μmol) or DMSO for 1 h. Then, these extracted proteins were treated with pronase E (20 μg/mL, MCE, USA) for 5 minutes at room temperature. WB was performed for each sample.

WB analysis

Cellular proteins were isolated with RIPA buffer, quantified by BCA assay (Beyotime, China) and denatured in loading buffer at 100°C for 10 minutes. Then, the samples were separated by SDS-PAGE and transferred onto a PVDF membrane. Next, the membrane was blocked with 5% skim milk at room temperature for 1 hour. The blots were incubated overnight at 4°C with primary antibodies against AKT (1:1000, CST, USA), phospho-AKT (Ser473) (1:1000, CST, USA), mTOR (1:1000, proteintech, USA), phospho-mTOR (Ser2448) (1:1000, proteintech, USA) and GAPDH (1:2000, proteintech, USA). After washing, secondary antibodies (1:2000, Proteintech, USA) were applied for 1h. Signals were developed using an ECL reagent and imaged on a Bio-Rad system (BioRad, CA, USA). Uncropped blots are available in Supplemental Information.

Cell counting Kit-8 (CCK-8) assay

We seeded CRC cells in a 96-well plate (2,000 cells per well) and treated them with quercetin (20 μmol) or DMSO for 24-hours. Detect cell proliferation every 12 hours for the next 48 hours. Next, add 10 ul CCK-8 reagent to each well, incubate at 37°C for 1 hour and then measure the absorbance at 450 nm.

Colony-formation assay

A total of 2,000 Caco2 and SW480 cells per well were seeded in a 6-well plate for the colony formation assay. After 7 days of quercetin (20 μmol) or DMSO treatment, the colonies were fixed (4% formaldehyde for 15 minutes), stained (0.5% crystal violet) and imaged as previously reported (Xu *et al*., 2024).

Scratch test

We performed a scratch test to assess CRC cell migration. SW480 and Caco2 cells were grown to high confluence (90-95%) in a 24-well plate before a scratch was made with a pipette tip. Post-washing with PBS to remove debris, cells were maintained in serum-free medium under different treatments. Then, the wound areas were imaged at 0 h and 24 h post-scratching on a fluorescence microscope (ThermoFisher Scientific, USA).

Transwell invasion assay

Transwell invasion assay was applied to test the invasive capacity of Caco2 and SW480 cells. As previously described (Xu *et al*., 2024), 2.5×10^4 SW480 cells in 200 μL serum-free medium were cultured in the Matrigel-coated upper chamber (Corning, USA) and 750 μL complete culture medium containing 10% FBS was filled in lower chamber. After 48 hours of incubation at 37°C, the non-invading cells on the upper surface of the membrane were removed. The cells that had invaded through the Matrigel and membrane to the lower surface were fixed, stained and imaged.

Statistical analysis

SPSS 21.0 software was used for all statistical analyses in this study. An independent sample test was used to compare two groups. A P-value < 0.05 was considered statistically significant.

RESULTS

The drug–compound–disease target network construction

A total of 11563 CRC-related targets were identified from 5 disease retrieval databases (Fig. 1A). Then, we screened 21 active compounds from Huanglian decoction (Table S1) and their 198 potential drug targets via TCMSP database. The intersection between the HD and CRC targets yielded 102 active compound–disease interaction targets (Fig. 1B). Fig. 1C shows the drug–compound–disease target network for HD. The observation that over half of the drug targets were CRC-related suggested that HD may possess anti-tumor properties.

GO and KEGG enrichment analysis of drug target genes

To investigate the putative therapeutic mechanisms of Huanglian decoction in CRC, we performed functional enrichment analysis on these active compound–disease interaction targets. The most significantly enriched biological processes (BP) were cellular response to chemical stress, epithelial cell proliferation and response to reactive oxygen species (Fig. 2A). Regarding cellular components (CC), the most enriched CC were plasma membrane raft, apical part of cell and spanning component of plasma membrane (Fig. 2B). Furthermore, most related molecular functions (MF) were ligand-activated transcription factor activity, nuclear receptor activity and transcription coactivator binding (Fig. 2C). Additionally, KEGG pathway enrichment analysis (excluded “Human Diseases” category) revealed that these targets are primarily involved in the MAPK signaling pathway, PI3K-Akt signaling pathway and cellular senescence (Fig. 3). These results indicated that Huanglian decoction may exert therapeutic effects in CRC development by regulating epithelial cell proliferation associated with signaling pathways such as PI3K-AKT.

Construction and analysis of PPI network

To identify key node targets underlying the anti-CRC mechanisms of HD, a PPI network was constructed by uploading the 102 active compound–disease interaction targets to the STRING database. Subsequently, using Cytoscape 3.10.3 and the cytoNCA plugin, 7 CRC related targets (AKT1, ESR1, JUN, IL6, MYC, FOS and CCND1) showed higher topology parameter scores and were considered as the core nodes (Fig. 4). These core targets are likely pivotal in mediating the therapeutic effect of Huanglian decoction.

AKT1 is a key target of quercetin

To validate the aforementioned findings, molecular docking between core targets and corresponding active compounds was conducted. The binding free energies ranged from -5.7 to -9.8 kcal/mol (Table 1), with values below -5.0 kcal/mol generally indicative of significant binding affinity in molecular docking. Notably, quercetin exhibited stable binding to all core targets of Huanglian decoction (HD) (Table 1), indicating that it may serve as a key therapeutic constituent underlying the efficacy of HD.

Quercetin-AKT1 exhibited the lowest binding free energy (-9.8 kcal/mol < -7.0 kcal/mol) and formed three hydrogen bonds, suggesting their robust binding ability. This interaction model is visualized in Fig. 5A. To validate AKT1 as a direct target of quercetin, CETSA was performed. Results revealed significantly increased thermal stability of AKT1 protein in quercetin-treated group compared to the control (Fig. 5B, Fig. S1). Further, the DARTS assay was also performed. Results showed higher protein level of AKT1 in quercetin-treated group compared to GAPDH group (Fig. 5C, Fig. S2), indicating that quercetin enhanced the tolerance of AKT1 protein to pronase. In summary, our data indicated that AKT1 is a key target of quercetin.

Quercetin suppressed CRC progression in-vitro by inhibiting AKT pathway activation

Human CRC cell lines, Caco2 and SW480, were used to explore the effects of quercetin on CRC progression *in vitro*. Treatment with quercetin did not change the protein level of AKT and mTOR but significantly decreased their phosphorylation level (Fig. 6A-B, Fig. S3, Fig. S4). The CCK-8 assay showed that quercetin inhibited proliferation in Caco2 and SW480 cells (Fig. 6C-D). Fig. 6E-F suggested that quercetin inhibited the tumorigenic potential of CRC cells (Caco2 and SW480). The scratch test results demonstrated that quercetin suppressed CRC cell migration (Fig. 6G-H). Moreover, trans well invasion assay found that quercetin decreased the invasion activity of SW480 (Fig. 6I). Collectively, these findings suggested that quercetin suppressed CRC progression via inhibition of the AKT pathway.

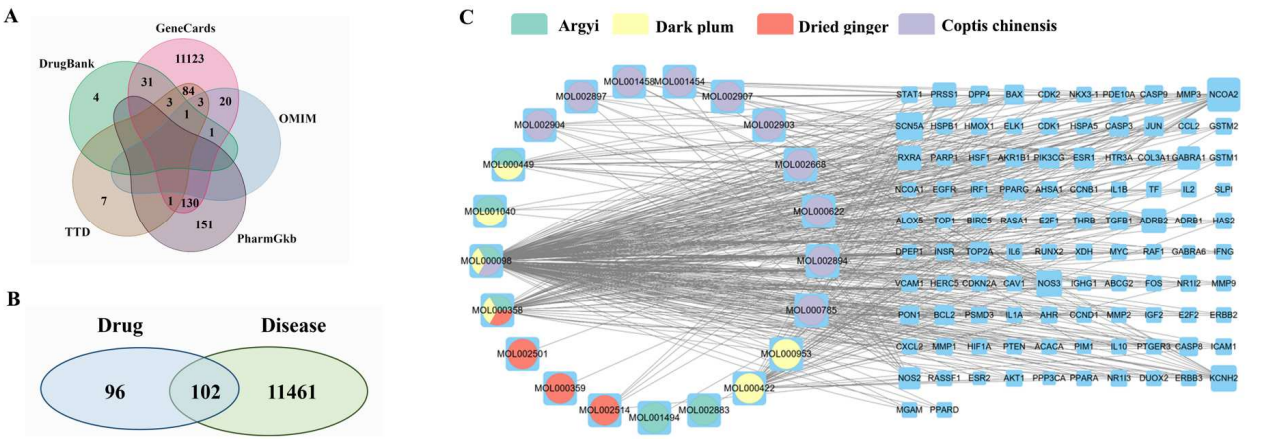

Fig. 1: Network pharmacology analysis. (A) Targets related to colorectal cancer; (B) Venn diagram of potential targets of huanglian decoction against colorectal cancer; (C) The drug-compound-disease target network of huanglian decoction against colorectal cancer.

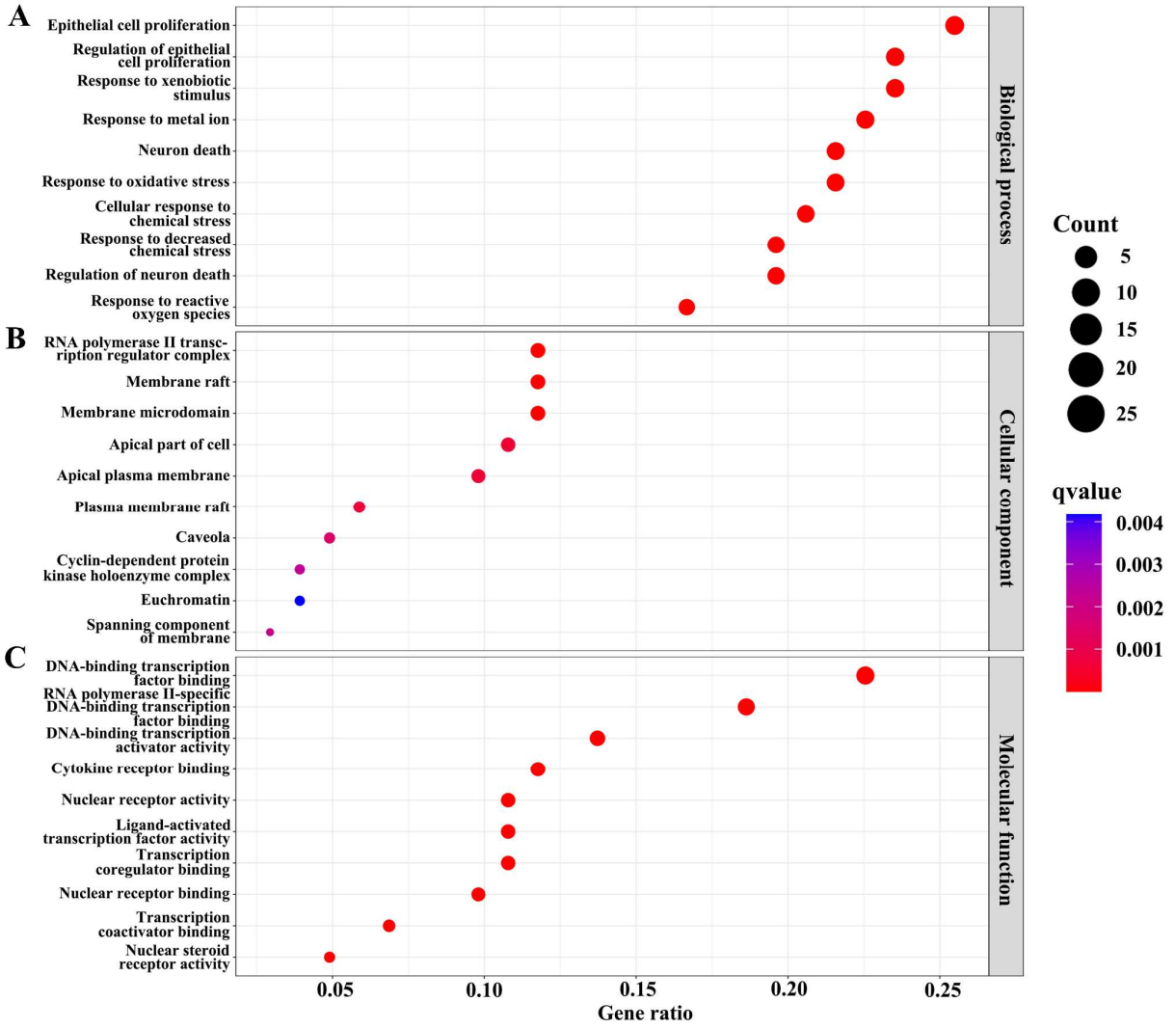

Fig. 2: GO enrichment analyses bubble diagram. (A) Top 10 items enriched in biological process; (B) cellular components; (C) molecular function.

Table 1: Binding energy for active compounds with their targets.

Active compound	PubChem ID	Target	Protein ID	Binding energy (kcal/mol)
Berberine	2353	ESR1	1ERR	-7.7
Berberrubine	72704	ESR1	1ERR	-7.9
Beta-sitosterol	222284	JUN	5T01	-6.3
Coptisine	72322	ESR1	1ERR	-8.7
Epiberberine	160876	ESR1	1ERR	-7.6
Kaempferol	5280863	AKT1	3O96	-9.5
Kaempferol	5280863	JUN	5T01	-5.8
Palmatine	19009	ESR1	1ERR	-7.2
Quercetin	5280343	CCND1	2W96	-7.3
Quercetin	5280343	IL6	1ALU	-7.1
Quercetin	5280343	MYC	1NKP	-6.7
Quercetin	5280343	FOS	1FOS	-5.8
Quercetin	5280343	AKT1	3O96	**-9.8**
Quercetin	5280343	JUN	5T01	-5.7
Worenine	20055073	ESR1	1ERR	-8.4

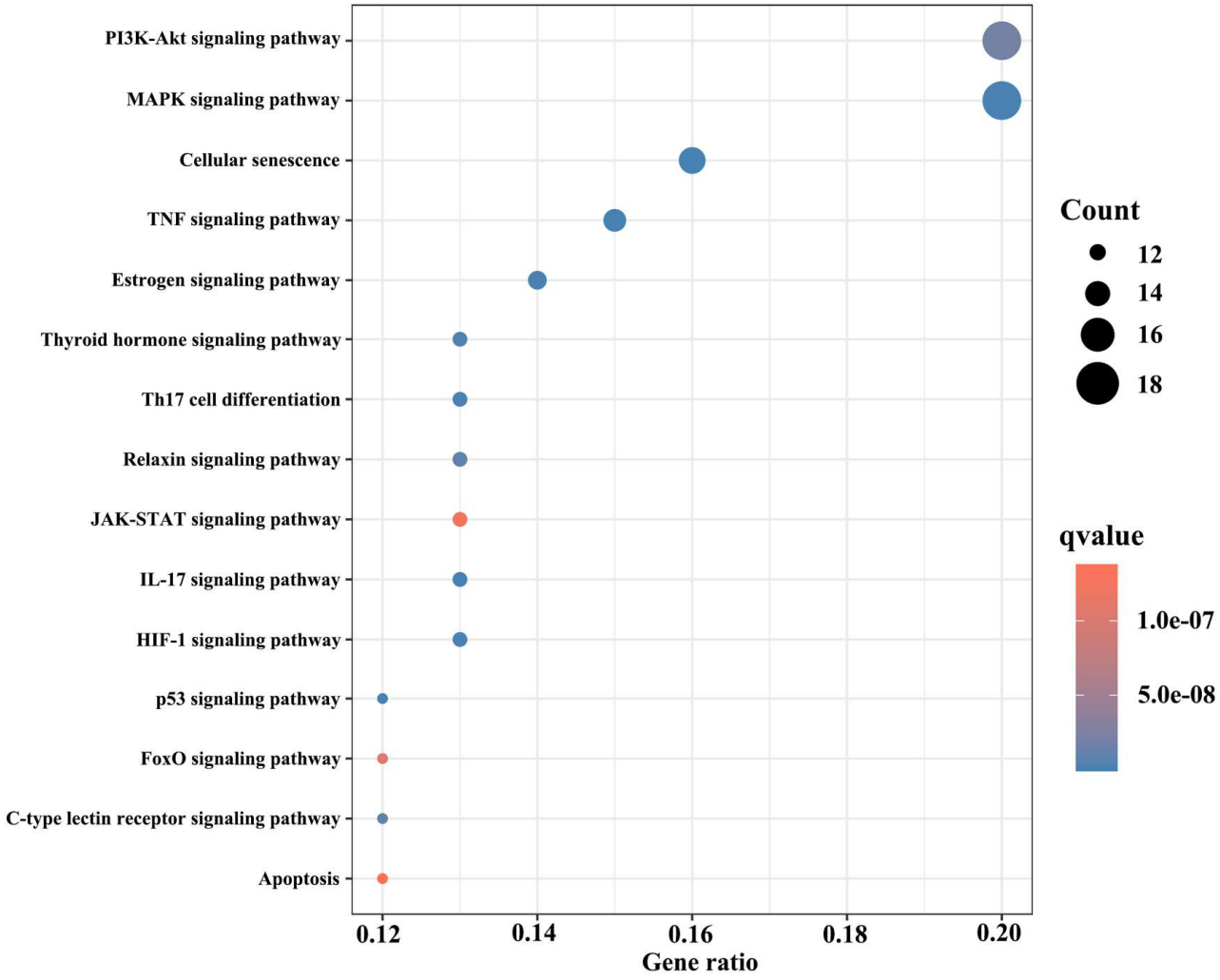

Fig. 3: KEGG enrichment analyses bubble diagram. Top 15 items enriched in KEGG.

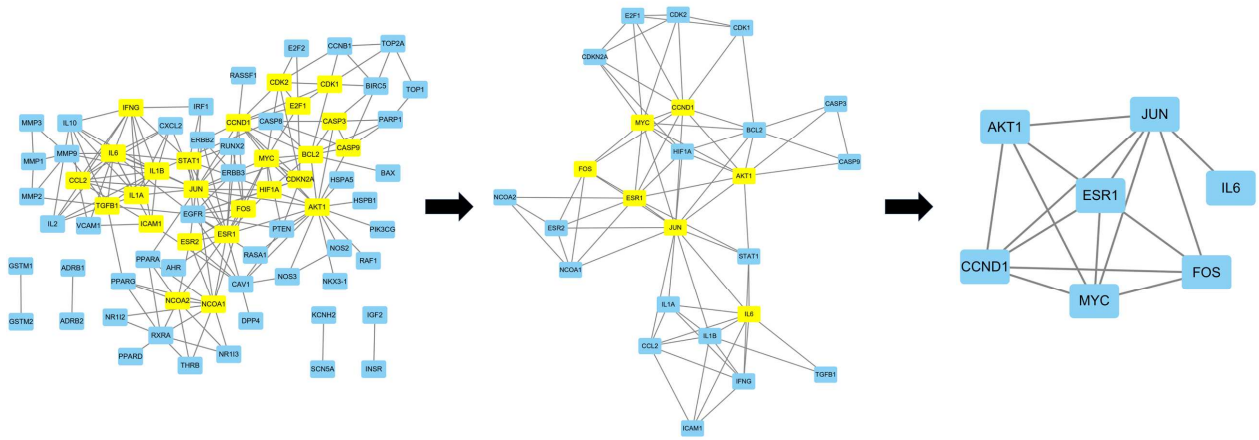

Fig. 4: Protein–protein interaction (PPI) network topological analysis. A primary PPI network of huanglian decoction and colorectal cancer common targets was generated using STRING. Then, a core PPI network was generated and visualized using the cytoNCA plugin in Cytoscape software.

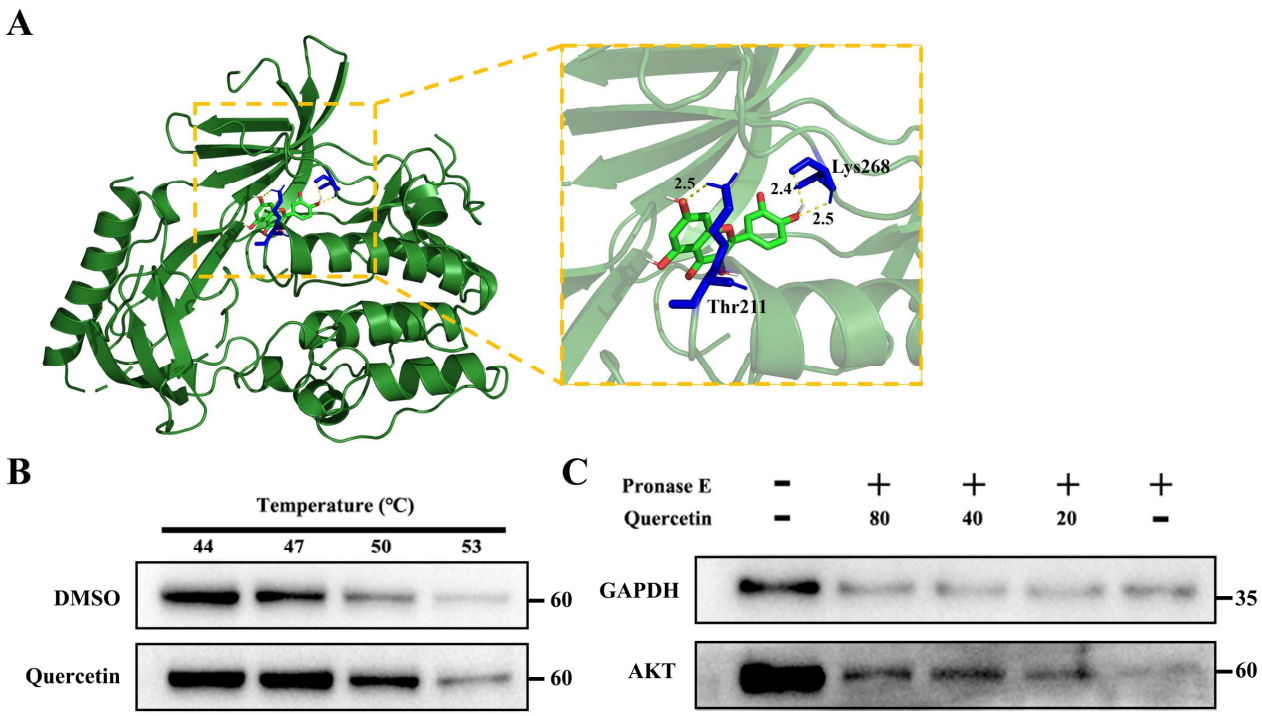

Fig. 5: AKT1 is a target of quercetin. (A) Schematic diagram of molecular docking of quercetin with AKT1; (B) WB detection of CETSA samples; (C) WB detection of DARTS assay samples.

DISCUSSION

CRC is the third most common tumor globally, with a rising tendency in recent years, particularly in early-onset CRC (Kong *et al*., 2023). In 2022 alone, it was responsible for 90.3 thousand fatalities (Chen *et al*., 2025b). TCM has long played a pivotal role in cancer management in ancient China and its clinical efficacy and low toxicity have been widely confirmed throughout its extensive historical practice (Li *et al*., 2025). HD is a TCM formula used to manage intestinal disorders. However, no studies to date have revealed whether HD exerts anti-CRC effects or the specific signaling pathways and regulatory mechanisms involved. Here, we employed a network pharmacology approach to identify potential targets of HD against CRC. Molecular docking and *in-vitro* experiments were further performed to filter the potential core active compound and investigate the impact of core compound on the biological behaviors of CRC cell lines.

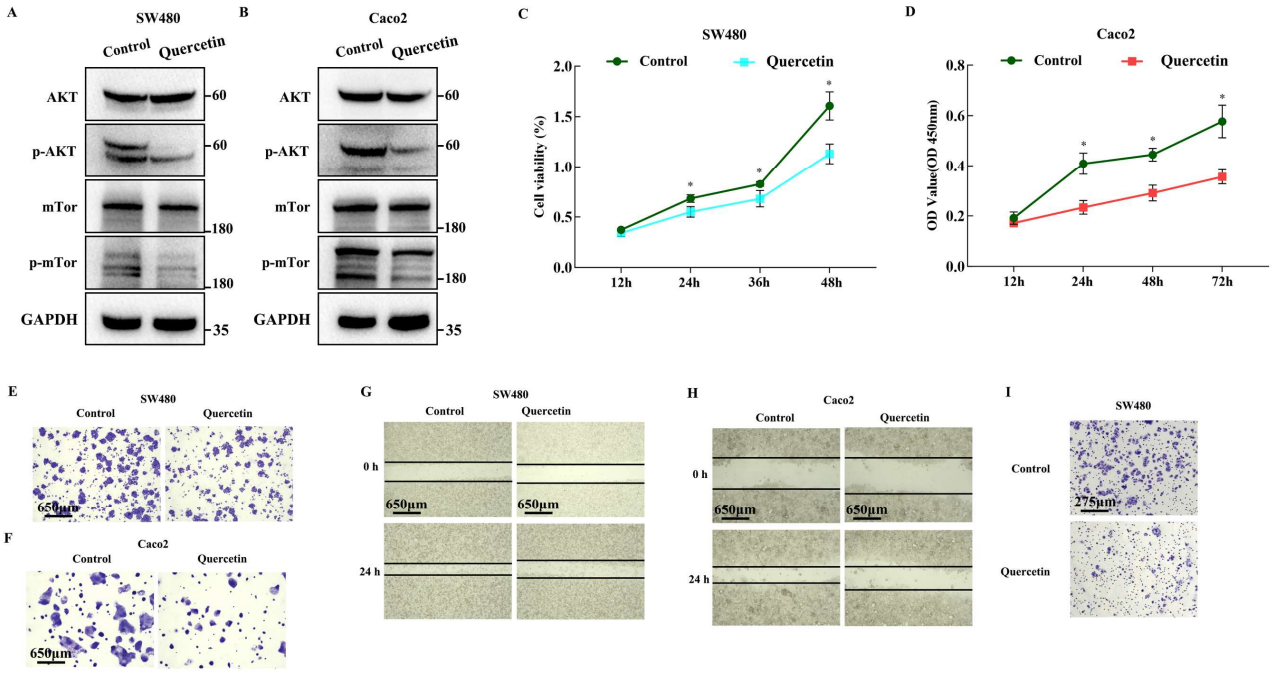

Fig. 6: Quercetin suppressed CRC progression *in-vitro* by inhibiting AKT signaling pathway activation. (A-B) Protein expression of p-AKT, AKT, p-mTOR and mTOR in Caco2 and SW480 cells after treating with quercetin (The membranes were cropped at indicated region specified in Supplementary Information); (C and D) The biological functions of quercetin on human CRC cell lines were validated by CCK-8; (E and F) Colony-formation assay; (G and H) Scratch test (I) Transwell invasion assay. *P < 0.05.

Bioinformatic profiling identified approximately 50% targets of HD were CRC-associated genes. Further KEGG pathway analysis demonstrated significant enrichment of these targets in various tumor diseases (e.g., bladder cancer, prostate cancer, CRC and lung cancer) (data not shown). The drug–compound–disease target network of HD found many active compounds, such as beta-sitosterol, berberine and quercetin. Beta-sitosterol, a phytosterol, has demonstrated efficacy in inhibiting proliferation of multiple cancer cell lines (Jin *et al*., 2025). Animal studies found that dietary supplementation with beta-sitosterol significantly depressed tumor metastasis (Jin *et al*., 2025). When combined with gemcitabine, β-sitosterol enhanced chemosensitivity of pancreatic cancer cells (Cao *et al*., 2018). Berberine is an isoquinoline alkaloid from *Coptis chinensis* and its anticancer properties have been reported across various tumors (Chen *et al*., 2025a). Modern pharmacological studies revealed that berberine can suppress carcinogenesis progression by promoting apoptosis, regulating cellular recycling and modulating gut microbiota (Chen *et al*., 2025a). In HepG2 and MCF7 cells, berberine downregulated the Akt/mTOR/GLUT1 signaling axis thereby effectively attenuating the Warburg effect, which is vital for tumor rapid proliferation and survival (Guo *et al*., 2021). Furthermore, PPI network identified seven potential targets (including AKT1, ESR1, JUN, IL6, MYC, FOS and CCND1) of HD that may be core target genes in CRC treatment. JUN and FOS are key subunits of the AP-1, which is related to tumor invasion, migration and epithelial mesenchymal transition (EMT) (Song *et al*., 2023). It has been revealed that *c*-Jun reprograms ERα-chromatin binding, regulates TGFB1 expression, thereby reducing tamoxifen sensitivity in ER^+ breast cancer cells (He *et al*., 2018). Chronic inflammation has been acknowledged as a canonical cancer hallmark and IL6 represents a critical cytokine in the crosstalk between inflammation and cancer (Soler *et al*., 2023). In oral squamous cell carcinoma, tumor-associated macrophages increased the stemness of tumor cells via the IL6/Stat3/THBS1 feedback loop (You *et al*., 2022). These evidences supporting the anti-CRC potential of HD.

Then, intriguing results revealed that quercetin exhibits potential to form stable complexes with all identified core proteins, suggesting it may be the principal active compound in HD. Quercetin is a naturally occurring flavonoid ubiquitously distributed in vegetables and fruits, possessing immunomodulatory, antioxidant and anti-inflammatory properties (Deng *et al*., 2025). Numerous studies have indicated that quercetin hampers tumor progression by inhibiting proliferation, suppressing angiogenesis, inducing apoptosis and enhancing chemo-sensitization (Deng *et al*., 2025). Our *in-vitro* findings align with prior findings (Cattivelli *et al*., 2023), showing that quercetin inhibited tumor cell proliferation. Moreover, quercetin inhibited the migration and invasion activities of CRC cells (Caco-2, SW480). KEGG enrichment analysis underscored the pivotal role of the PI3K-AKT pathway in

CRC treatment (Fig. 3). Previous studies characterized quercetin as a phosphatidylinositol 3-kinase (PI3K) inhibitor that potently suppresses PI3K activity (Navarro-Nunez *et al*., 2010). Our computational analysis further indicates that AKT1, a downstream protein of PI3K, exhibited the most stable binding to quercetin, validated by CETSA and DARTS assays. This suggests quercetin may directly target AKT1 independently of PI3K to exert its effects. Full activation of AKT1 requires phosphorylation at Thr308 and Ser473 (Alessi *et al*., 1997; Sarbassov *et al*., 2005). Phosphorylated AKT1 regulates several critical cellular functions, including cell proliferation, survival and neovascularization and its dysregulation has been reported in diverse tumors (Gehringer *et al*., 2020). In both Caco-2 and SW480 cell lines, quercetin reduced phosphorylation levels of AKT and its key downstream effector mTOR. These findings suggested quercetin may exert anti-CRC effects by directly targeting AKT1 to inhibit the AKT/mTOR pathway.

However, this research has limitations: the findings primarily rely on public databases and lack experimental validation of HD. Given the complexity of medicine formulas where various compounds may display synergistic or antagonistic interactions, focusing on key active components facilitates mechanistic exploration and clinical translation. Currently, only one representative constituent was investigated, which cannot fully reflect HD's therapeutic potential. Future studies exploring additional components coupled with *in-vivo* and clinical validation will enhance the credibility and translational value of these results.

CONCLUSION

Here, we used network pharmacology to elucidate the therapeutic potential of HD in CRC. Quercetin was identified as the most important active constituent, directly targeting AKT1 to suppress CRC cell proliferation and migration.

Acknowledgments
We would like to thank TCMSP, OMIM, DrugBank, STRING, PDB, PharmGKB and KEGG databases for the availability of the data.

Authors' contributions
Xusan Xu; Data curation, methodology, writing – review and editing and conceptualization; Yongjian Ye: Formal analysis, funding acquisition, investigation and writing–original draft; Xiaoxia Wang: Formal analysis, funding acquisition and writing–original draft.

Fundings
This work was supported by the Clinical and Translational Research Project of Anhui Province (202427b10020110), the Construction Project of the Institute of Pediatric Hemato-Oncology of Shunde Women and Children's Hospital (2024LCYJS002) and Medical Research Projects of Foshan (20240224).

Data availability statement
The datasets analysed in this study are available in several databases. These include the Therapeutic Target database (https://db.idrblab.net/ttd/), the Traditional Chinese Medicine Systems Pharmacology (TCMSP, https://www.91tcmsp.com/#/database) database, OMIM database (https://www.omim.org), DrugBank database (https://go.drugbank.com/), GeneCards database (https://www.genecards.org/), the STRING database (https://string-db.org/), the PDB database (https://www.rcsb.org/), the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), the Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.kegg.jp/kegg/kegg1.html) and PharmGKB database (https://www.pharmgkb.org/). All data is publicly available.

Ethical approval
The data for this study were obtained from the publicly available dataset and only commercially available established cell lines were used, so ethical approval was not required for this study in accordance with the local legislation and institutional requirements.

Conflict of interest
The authors declare no conflict of interest.

Supplementary data
https://www.pjps.pk/uploads/2026/06/SUP1782646429.pdf

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