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Research article

# Effect of Shan Zha (Hawthorn or Crataegus) on gastrointestinal cancer: A network pharmacology and molecular docking study

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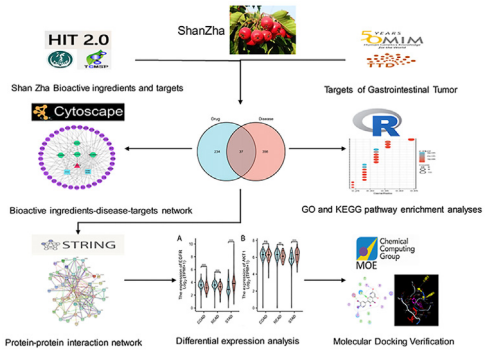
## HIGHLIGHTS

- Four main bioactive compounds were identified in the Shan Zha (hawthorn or Crataegus) extract.
- Drug (271) and gastrointestinal-tumor (393) targets of Shan Zha were identified.
- Quercetin and kaempferol showed promising binding for tumor protein p53 (TP53), cyclin D1 (CCND1), epidermal growth factor receptor (EGFR), and vascular endothelial growth factor A (VEGFA).
- Shan Zha affects gastrointestinal cancer through multiple target and pathway mechanisms.

## GRAPHICAL ABSTRACT




Workflow of the network pharmacology analysis.

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## ABSTRACT

*Background:* Shan Zha (Hawthorn or Crataegus) is a traditional Chinese medicine (TCM) most commonly used for the treatment of hyperlipidemia. Gastrointestinal cancer is closely correlated with blood lipid levels. This study illustrates the potential anticancer effects of Shan Zha on gastrointestinal tumors based on network pharmacology and molecular docking.

*Methods:* Hawthorn's bioactive ingredients and drug targets were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), Integrative Pharmacology-based Research Platform of Traditional Chinese Medicine version 2.0 (TCMIP v2.0), and Herbal Ingredients' Targets Platform (HIT 2.0) databases. Validated disease targets of gastrointestinal cancer were obtained from the Therapeutic Targets Database (TTD) and HIT 2.0 databases. Protein–protein interaction analysis of intersecting genes was performed using the Search Tool for the Retrieval of Interacting Genes (STRING) database. The functions of these genes were further analyzed by performing gene ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. Molecular docking verification was performed using Molecular Operating Environment (MOE) software.

*Results:* Four main bioactive components were identified in Shan Zha. A total of 271 potential drug targets were identified, and 393 gastrointestinal-tumor targets were obtained. Through protein interaction analysis of

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intersecting targets, the main components of Shan Zha were found to interact more closely with proteins such as tumor protein p53 (TP53), AKT serine/threonine kinase 1 (AKT1), *JUN* proto-oncogene (JUN), interleukin 6 (IL6), epidermal growth factor receptor (EGFR), and vascular endothelial growth factor A (VEGFA). KEGG pathway enrichment analysis showed a total of 127 pathways, mainly involving pathways in multiple types of cancer, the Phosphatidylinositol 3-kinase-Akt (PI3K-Akt) signaling pathway, and EGFR tyrosine kinase inhibitor resistance. Combined with The Cancer Genome Atlas (TCGA) differential analysis, key targets, including *TP53*, cyclin D1 (CCND1), EGFR, and VEGFA, were screened. Molecular docking results showed that quercetin and kaempferol had the good binding potential for TP53, CCND1, EGFR, and VEGFA.
*Conclusion:* These findings suggest that Shan Zha exerts its effects on gastrointestinal cancers through a multi-target, multi-component, and a multi-pathway mechanism.

## Introduction

Shan Zha (also known as hawthorn or Crataegus) is a well-known traditional Chinese medicine (TCM), and its different varieties are used worldwide. It is typically used to promote digestion and improve appetite. More than 100 compounds, especially phenolic compounds, have been identified in Shan Zha.[1] Hawthorn exhibits a protective effect against atherosclerosis in apolipoprotein E-deficient mice, and the mechanism may be related to the improvement of blood lipids and antioxidant effects.[2] Three main antithrombotic components were isolated from the crude extract of hawthorn leaves, which have both antiplatelet aggregation and antithrombotic effects.[3] Hawthorn has been studied for its role in regulating blood lipid levels and protecting the heart. The clinical benefit of hawthorn in chronic congestive heart failure (CHF) has been well documented.[4] A meta-analysis of eight trials, including 632 patients with CHF, showed that hawthorn extract administered as an adjunctive therapy had a significant benefit compared with the placebo and significantly improved symptoms of dyspnea and fatigue.[5] Shan Zha is the most commonly used single TCM for the treatment of hyperlipidemia.[6] Additionally, hawthorn is used to treat dyslipidemia, hypertension, angina pectoris, and arrhythmia.[7,8]

The incidence of hyperlipidemia and hypercholesterolemia is gradually increasing with improvements in people's standards of living and lifestyle changes.[9] Hyperlipidemia and related diseases are common worldwide. The relationship between hyperlipidemia and tumor development has also been increasingly confirmed. Apolipoprotein A1 and lower serum high-density lipoprotein cholesterol levels may be associated with an increased risk of breast cancer.[10] Patients with chronic-viral hepatitis often exhibit abnormal lipid metabolism.[11] There is also a close relationship between blood lipid levels and liver cancers.[12] A prospective study found that higher concentrations of serum high-density lipoprotein, a lipid that is generally considered protective, are associated with a lower risk of colon cancer.[13] Disorders and abnormal changes in lipid levels in the cancer tissues and sera of patients with colorectal cancer may be correlated with the occurrence and development of colorectal cancer.[14] Gastrointestinal tumors may be closely associated with nutrient metabolism.[15,16] Hawthorn can also be used to improve the dyspepsia of patients with cancer owing to its digestive-stimulating effect.[17] A recent study found that hawthorn may inhibit breast cancer growth at the stem cell level, regardless of hormone dependency.[18] It also inhibited cell proliferation and induced apoptosis in hepatocellular carcinoma.[19,20] The potential mechanism of action of hawthorn in gastrointestinal cancer has not been investigated. Therefore, by performing network pharmacology and molecular docking studies, combined with related studies on lipid metabolism and tumors, we expounded on the potential anticancer effects of Shan Zha in gastrointestinal-malignant tumors.

## Methods

### *Shan Zha bioactive ingredients and target prediction*

The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP),[21] Integrative Pharmacology-based Research Platform of Traditional Chinese Medicine version 2.0 (TCMIP v2.0),[22] and Herbal Ingredients' Targets Platform (HIT 2.0)[23] databases were used to predict the bioactive ingredients in Shan Zha. The pharmacokinetic parameters of the components, oral bioavailability (OB) and drug-likeness (DL), were used as the screening conditions in this study as follows: OB $\geq$ 40% and DL $\geq$ 0.18.[21] These components were then screened using TCMIP quantitative estimation of drug affinity (QED score). The above components with moderate QED scores (0.49 $\leq$ QED $\leq$0.67) and good QED scores (QED >0.67) were retained.[22] The bioactive-component targets were searched for in the TCMSP and HIT 2.0 (http://www.badd-cao.net:2345/) databases.[23] TCMSP provides information on drugs that have been experimentally and clinically verified or marketed in the DrugBank database. The UniProt database (www.uniprot.org/)[24] was used to obtain compound molecular targets and gene identities. The HIT 2.0 database was used to complement the validated targets for each active ingredient. Duplicate values of related targets were merged and deleted to obtain drug targets.

### *Screening gastrointestinal-tumor targets*

Gastrointestinal tumor-related targets were acquired from the Therapeutic Targets Database (TTD) (http://db.idrblab.net/ttd/)[25] and Online Mendelian Inheritance in Man (OMIM) database (https://www.omim.org/).[26] The screening criteria were gastric, intestinal, and colorectal cancers. The obtained targets were combined into disease targets.

### *Bioactive ingredient-target network construction*

The intersection of drug and disease targets was selected to obtain 37 common target genes, and a bioactive ingredient-common target-disease network file was constructed. The file was imported into Cytoscape v3.9.1 software[27] to construct a bioactive ingredient-disease-target network diagram.

### *Protein–protein interaction network construction and analysis*

The Search Tool for the Retrieval of Interacting Genes (STRING) (https://string-db.org)[28] is a search tool for known and predicted protein–-protein interactions (PPI) based on public databases. The intersection target of the bioactive ingredient and gastrointestinal cancer was input into the multiple protein nodes of the STRING database. *Homo sapiens* was chosen as the organism; the minimum required interaction score was 0.7, and disconnected nodes were hidden in the network. An interaction network of common target genes of drugs and diseases was constructed. The PPI network was exported for protein interaction statistics.

### *Gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses*

The clusterProfiler package[29] of R software (version 3.6.3)[30] was used for enrichment analysis, with $p.adj < 0.05$ and $q < 0.2$ as the standards; gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed on the intersection targets. GO enrichment analysis included biological processes (BPs), cellular components (CC), and molecular functions (MF).

The top 15 entries of the GO and KEGG pathway enrichment analyses are presented in the form of histograms and bubble charts, respectively.

*The Cancer Genome Atlas database validation*

The RNA-seq data were downloaded in TPM (transcripts per million reads) format from TCGA (cancer tissue) and GTEx (healthy tissue) databases, which were processed using the Toil workflow from the University of California Santa Cruz (UCSC) Xena platform[31] (https://xenabrowser.net/datapages/), and log2 transformations were then performed for differential analysis. Next, the expression differences of hub genes in gastrointestinal cancers were compared with those in normal tissues.

*Molecular docking*

The reliability of the predicted targets was verified by molecular docking of the two core bioactive ingredients and four core targets screened. The 3D protein conformation of the core protein with crystal resolution below 3 Å, determined by X-ray diffraction, was downloaded in PDB format from the PDB database (https://www.rcsb.org/).[32] The 2D structure of the core components was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/)[33] in SDF format. The file was imported into the MOE (2019.01) software,[34] all water molecules were removed, the protein structure was prepared, the energy of small molecular components was minimized, molecular docking was performed, and docking scores were obtained. The MOE software was used to visualize the docking results and build a docking interaction–pattern diagram.

## Results

*Bioactive ingredients of Shan Zha and gastrointestinal tumor targets*

The 52 chemical constituents of hawthorn were identified using TCMSP, TCMIP v2.0, and HIT 2.0 databases. Four active compounds were screened: kaempferol, ent-epicatechin, quercetin, and isorhamnetin. The molecular identities are listed in Table 1. According to these four compounds, 204 drug targets verified by DrugBank were obtained from TCMSP, and 266 targets were obtained from HIT 2.0. After removing duplicates, 271 targets were screened. Screening criteria for the stomach, gastric, colon, and colorectal cancers were applied, and 152 cancer targets were identified. Using the OMIM database, 303 targets were screened for gastrointestinal neoplasms, and 393 disease targets were obtained by deleting duplicate values.

*Ingredients-target-disease network construction*

The intersection of drug and disease targets was used to construct a Venn diagram [Figure 1]; 37 common target genes were identified, and Cytoscape was used to construct a bioactive ingredient-common target-disease network diagram. Gastrointestinal tumors included stomach adenocarcinomas (STAD) and colorectal adenocarcinomas (COADREAD); the drug was shown as "SHAN ZHA," and the resulting network contained 44 points and 111 edges [Figure 2].

**Table 1**
Bioactive ingredients of Shan Zha.

| MOL ID | Bioactive ingredient | MW (g/moL) | OB% | DL | QED score |
|---|---|---|---|---|---|
| MOL000073 | Ent-epicatechin | 290.29 | 48.96 | 0.24 | 0.51 |
| MOL000098 | Quercetin | 302.25 | 46.43 | 0.28 | 0.51 |
| MOL000354 | Isorhamnetin | 316.28 | 49.60 | 0.31 | 0.67 |
| MOL000422 | Kaempferol | 286.25 | 41.88 | 0.24 | 0.64 |

DL: Drug-likeness; MW: Molecular weight; OB: Oral bioavailability; QED: Quantitative estimate of drug-likeness.

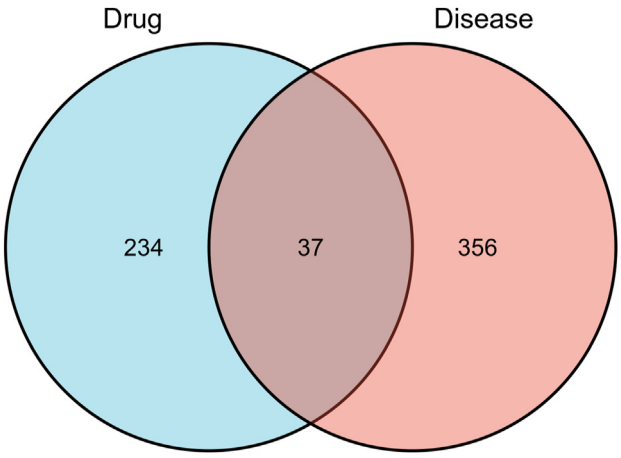


**Figure 1.** A Venn diagram showing the intersection of identified targets of drug bioactive ingredients and gastrointestinal-tumor genes. Area total of 37 common-target genes are identified.

*Construction and analysis of the protein–protein interaction network*

Intersection targets of bioactive ingredients and gastrointestinal cancers were input into the STRING database for PPI network construction, and the resulting network contained 37 points and 320 edges. Cytoscape was used to perform network analysis of the PPI file to form a network diagram [Figure 3A], which showed that each target exhibited a good interactive relationship. The larger the degree value in the figure, the darker the color; this indicates that more interactions are present in the network. 14 key targets were selected from the PPI network via the cytoHubba plugin of Cytoscape software, including *TP53*, *VEGFA*, *JUN*, *IL6*, *AKT1*, and *EGFR* [Figure 3B]. R was used to perform the statistical analysis and visualization of the correlation heat map of the 14 gastrointestinal-cancer hub genes [Figure 3C]. The coefficient of correlation between IL6 and PTGS2 $r = 0.718$, IL6 and IL1B $r = 0.69$, and SIRT1 and PTEN $r = 0.693$, which are moderately correlated. The other molecules are weakly correlated with each other.

*Gene ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses*

GO enrichment and KEGG pathway enrichment analyses of the intersection targets were performed using the clusterProfiler package in R software. Using $p.adj < 0.05$ and $q < 0.2$ as the standard values, GO enrichment analysis obtained 1704 BP, 27 CC, and 62 MF entries. Entries with significant BP enrichment mainly included epithelial cell proliferation, reproductive structure development, cellular response to abiotic stimuli, and cellular response to environmental stimuli. The top 15 entries were visualized using a column chart [Figure 4A]. KEGG pathway enrichment analysis identified 127 pathways, mainly involving human cytomegalovirus infection, human papillomavirus infection, proteoglycans in cancer, microRNAs in cancer, PI3K-Akt signaling pathway, *EGFR* tyrosine kinase inhibitor resistance, and several other cancer pathways [Table 2]. The top 15 pathways were selected to draw a bubble chart [Figure 4B]. Cytoscape was used for target-pathway network analysis. A target-pathway network diagram was constructed [Figure 4C]. It showed *PIK3CB, MAP2K1, EGFR, TP53, CDKN1A, CCND1, AKT1, BAX, PTEN*, and *CTNNB1* were the top ten genes selected according to the degree of association in the network. The top ten genes were all enriched in human papillomavirus infection (hsa05165), breast cancer (hsa05224), hepatocellular carcinoma (hsa05225), and endometrial cancer (hsa05213).

*The Cancer Genome Atlas database analysis*

The Cancer Genome Atlas (TCGA) (cancer tissue) and Genotype-Tissue Expression (GTEx) (healthy tissue) data downloaded from the

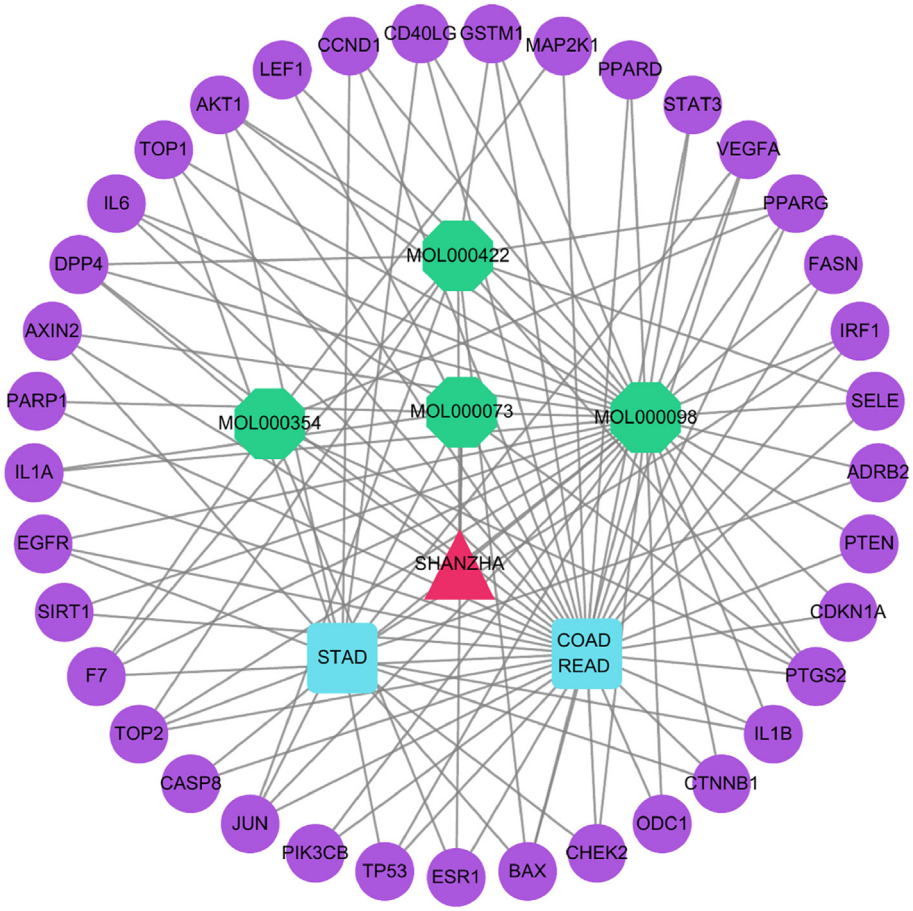


**Figure 2.** Bioactive ingredients-disease-targets network diagram. The purple circles represent genes, green octagons represent bioactive ingredients, blue rectangles represent diseases, and red triangle represents "SHAN ZHA."

UCSC Xena platform was used to compare the differences in the top eight hub genes between gastrointestinal and normal tissues by performing a Mann–Whitney *U* test [Figure 5]. The expression of *TP53*, *CCND1*, and *VEGFA* in tumor tissues was significantly higher than that in normal tissues ($P < 0.05$). *EGFR* was highly expressed in gastric cancer compared with that of normal tissue ($P < 0.001$); however, its expression in

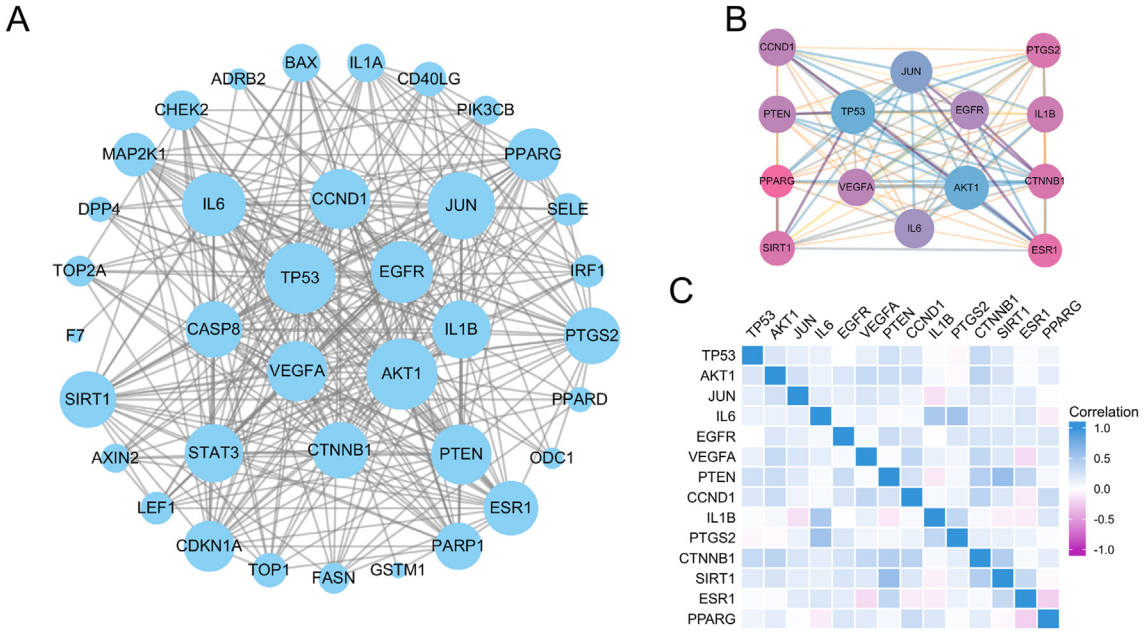


**Figure 3.** Protein–protein interaction network. (A) Protein–protein interaction network diagram of the intersected targets. (B) The 14 key target proteins were identified by protein interactions. (C) Heat map of the association of 14 genes in gastrointestinal cancer.

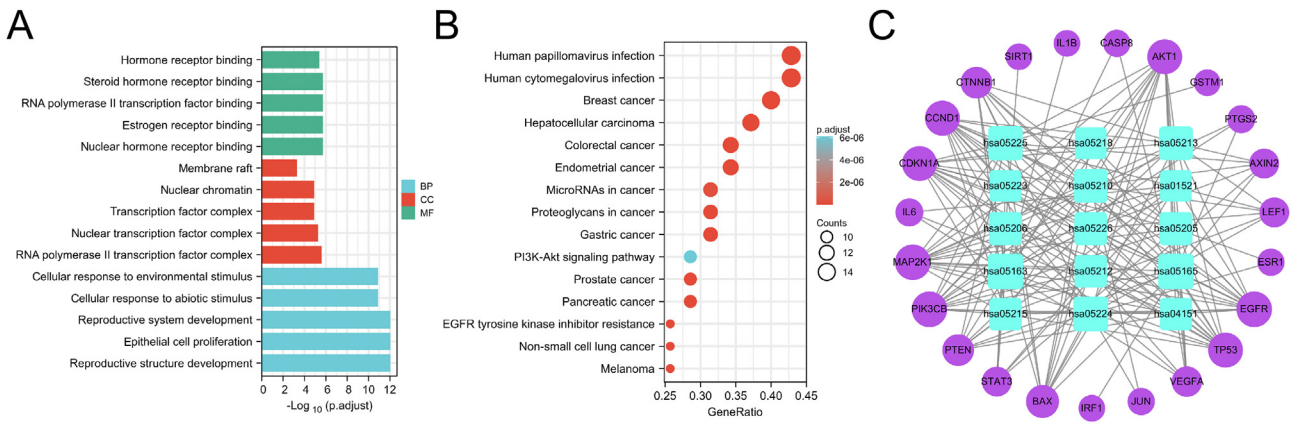


**Figure 4.** Gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses. (A) The column chart of the GO enrichment analysis. (B) The bubble chart of the KEGG pathway enrichment analysis. (C) Network diagram of genes-KEGG pathways. The size of the pathway with the blue rectangle showed the number of genes connected. GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

colorectal cancer was significantly lower than in normal tissues ($P < 0.001$). The differences in expression of the four genes in gastrointestinal tumors were significantly different when compared to that in normal tissues, but the levels were not the same.

### *Molecular docking*

Molecular docking simulates the interactions between small ligand molecules and receptor protein macromolecules. Molecular docking of the two core-active ingredients and four core-disease targets were performed. The basic information of the proteins corresponding to the targets, the score obtained by molecular docking, and the number of hydrogen bonds are shown in Table 3. The molecular docking results showed that quercetin and kaempferol had good docking potential with *TP53*, *CCND1*, *EGFR*, and *VEGFA*. Quercetin and kaempferol, and *TP53*, *CCND1*, *EGFR*, and *VEGFA* molecular docking model diagrams were visualized [Figures 6 and 7].

## Discussion

Four main bioactive ingredients were identified in hawthorn: kaempferol, ent-epicatechin, quercetin, and isorhamnetin. There are 37 common targets for the bioactive ingredients of drugs and gastrointestinal cancer. Through PPI network analysis, the main components of hawthorn were shown to interact more closely with 10 targets, which included *TP53*, *VEGFA*, *JUN*, *IL6*, *AKT1*, and *EGFR*. GO enrichment analysis showed that the BPs mainly included epithelial cell proliferation, reproductive structure development, cellular response to abiotic stimuli, and cellular response to environmental stimuli. KEGG pathway enrichment analysis mainly involved human cytomegalovirus infection, human papillomavirus infection, proteoglycans in cancer, miRNAs in cancer, the PI3K-Akt signaling pathway, *EGFR* tyrosine kinase inhibitor resistance, and several cancer pathways. Genes that play key roles in these pathways include *PIK3CB*, *MAP2K1*, *EGFR*, *TP53*, *CDKN1A*, and *CCND1*. Among the hub genes, four were identified to be differentially expressed in gastrointestinal tumors compared with that of normal tissues from the TCGA database. The molecular docking results showed that quercetin and kaempferol had good docking potential with *EGFR*, *CCND1*, *TP53*, and *VEGFA*, suggesting that quercetin and kaempferol may play a role in gastrointestinal cancers.

**Table 2**
The enrichment pathways corresponding to intersection genes.

| ID | Description | *p.adjust* | *q* | Count |
|---|---|---|---|---|
| hsa05163 | Human cytomegalovirus infection | 2.30867E-13 | 7.06737E-14 | 15 |
| hsa05165 | Human papillomavirus infection | 3.65224E-11 | 1.11803E-11 | 15 |
| hsa05224 | Breast cancer | 3.22486E-14 | 9.87203E-15 | 14 |
| hsa05225 | Hepatocellular carcinoma | 2.77967E-12 | 8.50919E-13 | 13 |
| hsa05213 | Endometrial cancer | 8.10346E-16 | 2.48065E-16 | 12 |
| hsa05210 | Colorectal cancer | 3.22486E-14 | 9.87203E-15 | 12 |
| hsa05226 | Gastric cancer | 2.03403E-10 | 6.22663E-11 | 11 |
| hsa05205 | Proteoglycans in cancer | 4.31197E-09 | 1.31999E-09 | 11 |
| hsa05206 | MicroRNAs in cancer | 2.16513E-07 | 6.62794E-08 | 11 |
| hsa05212 | Pancreatic cancer | 1.10124E-11 | 3.37116E-12 | 10 |
| hsa05215 | Prostate cancer | 9.94126E-11 | 3.04324E-11 | 10 |
| hsa04151 | PI3K-Akt signaling pathway | 6.09848E-06 | 1.86688E-06 | 10 |
| hsa05218 | Melanoma | 1.63027E-10 | 4.99061E-11 | 9 |
| hsa05223 | Non-small cell lung cancer | 1.63027E-10 | 4.99061E-11 | 9 |
| hsa01521 | EGFR tyrosine kinase inhibitor resistance | 2.75883E-10 | 8.44539E-11 | 9 |

AKT1: AKT serine/threonine kinase 1; EGFR: Epidermal growth factor receptor; PI3K: Phosphatidylinositol 3-kinase.

Gastrointestinal cancer is the most common malignant tumor worldwide.[35] The incidence of gastric and colorectal cancers in China ranks second and third, respectively, and the mortality rate ranks fourth and fifth, respectively, in the world.[36] Tumors are regarded as not only genetic and immune diseases but also metabolic diseases.[37] Metabolic reprogramming and changes in bioenergy are the hallmarks of cancer.[38] Overexpression of transporter genes may induce the invasion and migration of cancer cells.[39,40] Patients with a high apolipoprotein B/apolipoprotein A1 ratio have a shorter overall survival; this ratio can be used as a prognosis for gastric cancer.[41] Fatty acids have been identified as nutrients that cancer cells can rely on during metastatic colonization to increase their adenosine triphosphate (ATP) supply.[42] High blood lipid levels can promote the occurrence of distant metastasis in patients with colorectal cancer and is an indicator of the prognosis of metastatic colorectal cancer.[43,44] Hawthorn extract can significantly reduce body weight and serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL), and liver cholesterol levels in hyperlipidemic mice and can inhibit 3-hydroxy-3-methylglutaryl-coenzyme A Reductase (HMGCR) transcription in a high-fat diet.[45] Additionally, Shan Zha can reduce animal body weight mainly by activating Peroxisome Proliferator-activated Receptor-α (PPARα), improving dyslipidemia, reducing the levels of TC, TG, and LDL, and increasing the level of high-density lipoprotein.[46] Shan Zha extracts also significantly improved hyperlipidemia in mice fed a high-fat diet and increased the expression of hepatic PPARα. It can promote the degradation of β-oxidation-related enzymes in the liver by up-regulating PPARα expression, resulting in lower blood lipids.[47]

*EGFR* activation can trigger various downstream intracellular signaling pathways, including *RAS/RAF* and *PI3K/AKT*, to regulate cell

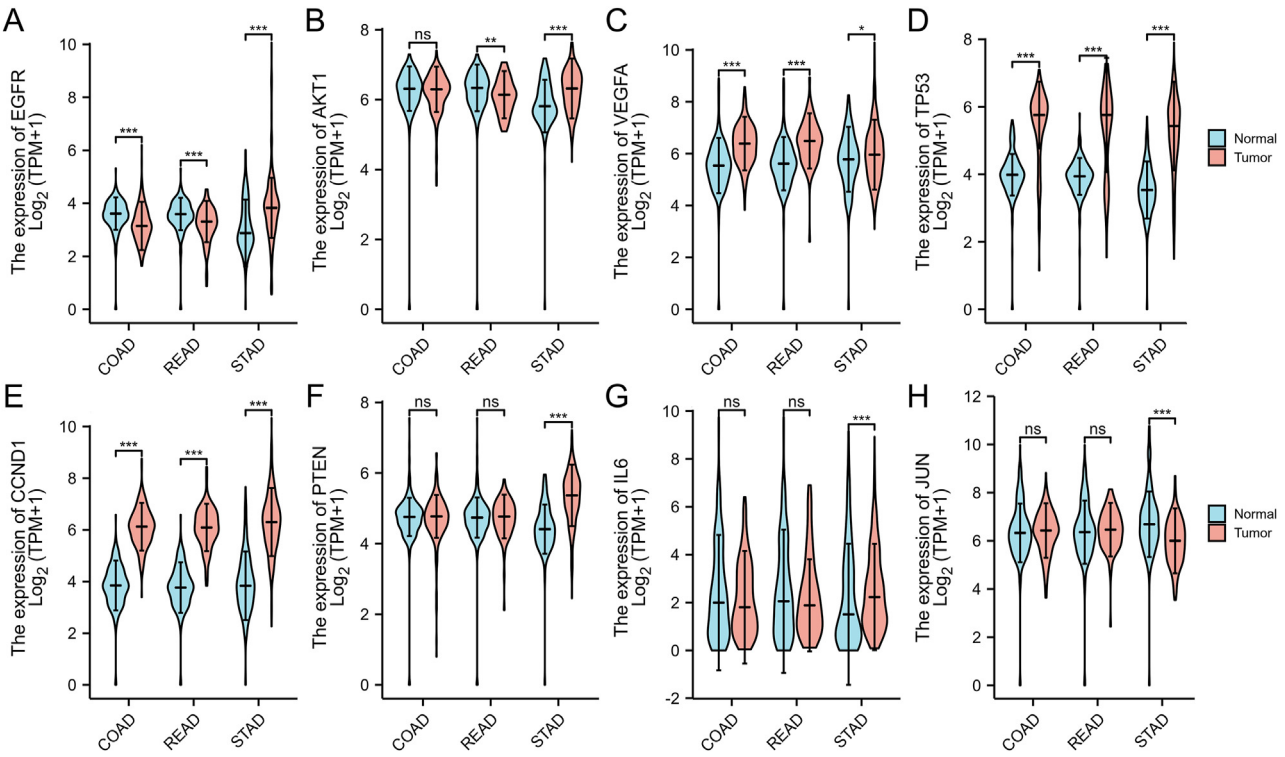


**Figure 5.** The differences in *EGFR*, *AKT1*, *VEGFA*, *TP53*, *CCND1*, *PTEN*, *IL6*, and *JUN* genes between gastrointestinal tumors and normal tissues from TCGA and GTEx data. *AKT1*: AKT serine/threonine kinase 1; *CCND1*: Cyclin D1; *COAD*: Colon adenocarcinoma; *EGFR*: Epidermal growth factor receptor; *IL6*: Interleukin 6; *JUN*: Jun proto-oncogene. *PTEN*: Phosphatase and tensin homolog; *READ*: Rectum adenocarcinoma; *STAD*: Stomach adenocarcinoma; *TP53*: Tumor protein p53; *VEGFA*: Vascular endothelial growth factor A. ns: $P \geq 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

growth, survival, and migration.[48] The vascular endothelial growth factor (*VEGF*) pathway is correlated with the angiogenesis pathway and plays a crucial role in the occurrence, growth, and metastasis of tumors.[49,50] Methods targeting *EGFR* and *VEGF* and related pathways, such as cetuximab and bevacizumab, have been widely used in clinical practice for cancer treatments.[51] *EGFR* can play a regulatory role in fat metabolism in adult male mice, which is positively correlated with elevated cholesterol levels.[52] *CCND1* encodes cyclin D1, which is an oncogene thought to be a driver of solid tumors and hematologic diseases.[53] *CCND1* belongs to a family of highly conserved cell-cycle proteins that are frequently overexpressed in a variety of malignancies such as hepatocellular carcinoma (HCC)[54] and breast cancer, and its overexpression is associated with tumor proliferation, migration, metastasis, and prognosis.[55,56] *TP53* is one of the most well-known tumor suppressor genes, and its amplification in gastric cancer is associated with abnormal cholesterol anabolism.[57] Mutations in *TP53* can lead to increased gene expression of key enzymes involved in fatty acid and cholesterol biosynthesis and inhibit fatty acid oxidation, thereby promoting lipid synthesis and accelerating tumor growth and progression.[58] Therefore, hawthorn has a potential inhibitory effect on gastrointestinal tumors by regulating BPs, such as proliferation, apoptosis, and oxidative stress, and may be correlated with cholesterol metabolism.

Hawthorn extracts also exert antioxidative effects and inhibit inflammation. By regulating oxidative stress and inhibiting inflammation, the expression of inflammatory markers such as interleukin-1, tumor necrosis factor-α, transforming growth factor-β1, nuclear factor-kappa-B, and cyclooxygenase-2 can be downregulated; therefore, it has a protective effect on liver fibrosis and effectively reduces inflammation and oxidative stress in rats and mice.[59,60] These results are consistent with the BPs of the main components of hawthorn in response to hypoxia which was observed by GO and KEGG analyses, and the possible oxidative stress effects mediated by the *HIF-1* signaling pathway(hsa04066).

The components of hawthorn can effectively inhibit the tyrosinase-mediated melanin production of melanoma cells by inhibiting the oxidation of melanoma cells *in vitro* and can also effectively inhibit the growth of melanoma cells *in vitro*.[61] The petroleum ether extract of hawthorn is cytotoxic and can induce apoptosis.[62] The natural extract of hawthorn has strong anticancer properties, significantly altering the migration, proliferation, and adhesion of tumor cells, and can effectively enhance the sensitivity of tumor cells to doxorubicin.[63] Hawthorn extract can also inhibit tumors by disrupting cell cycle regulation. The main components of hawthorn can inhibit the progression of human breast cancer through cell cycle arrest (G1 and S phases) and can induce the activation of caspase-9 and caspase-3, which are involved in

**Table 3**
Molecular docking of four target proteins and four chemical compounds from Shan Zha.

| Target | Uniprot-ID | PDB-ID | Binding energy (kcal/moL) | | Number of hydrogen bonds | |
|---|---|---|---|---|---|---|
| | | | Quercetin | Kaempferol | Quercetin | Kaempferol |
| TP53 | P04637 | 4MZI | −4.79 | −4.64 | 3 | 3 |
| CCND1 | P24385 | 6P8E | −4.34 | −4.25 | 3 | 3 |
| EGFR | P00533 | 3W2R | −4.83 | −4.69 | 4 | 2 |
| VEGFA | P15692 | 3QTK | −5.07 | −4.60 | 3 | 2 |

CCND1: Cyclin D1; EGFR: Epidermal growth factor receptor; TP53: Tumor protein p53; VEGFA: Vascular endothelial growth factor A.

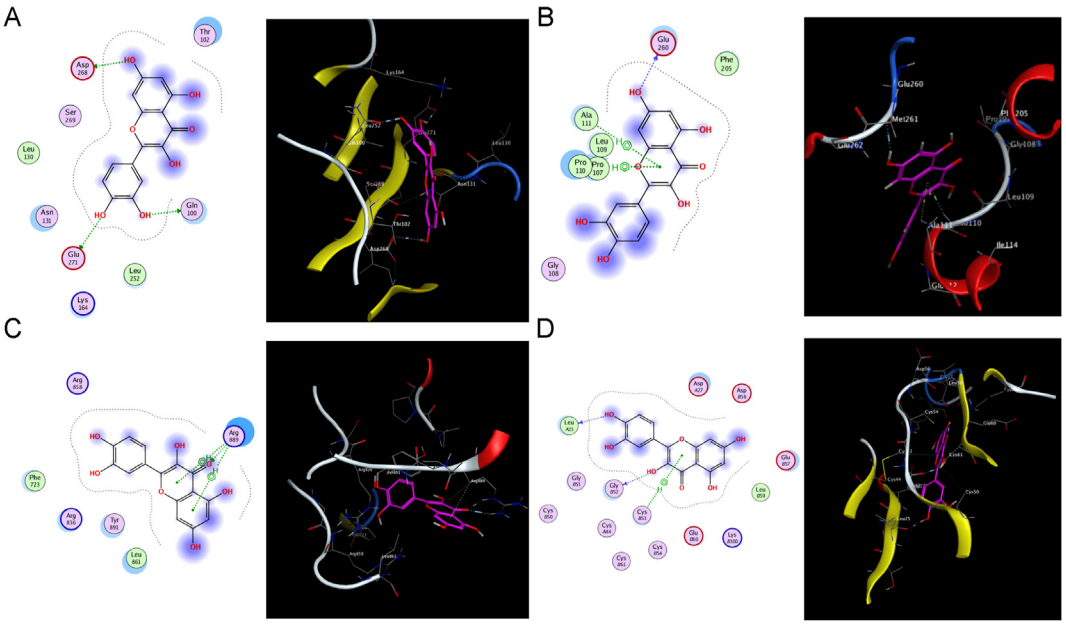


**Figure 6.** Diagram of molecular docking. The quercetin and TP53 (A), CCND1 (B), EGFR (C), and VEGFA (D) molecular docking model diagrams. CCND1: Cyclin D1; EGFR: Epidermal growth factor receptor; TP53: Tumor protein p53; VEGFA: Vascular endothelial growth factor A.

apoptosis.[64,65] The enrichment of biological functions was shown to include a regulatory function on the cell cycle (GO:2000045, GO:1902806). *CCND1*, which was identified among key targets, is a cell cycle protein. Moreover, the KEGG analysis provided a signaling pathway involved in apoptosis (hsa04210) due to its antioxidant effect in radiotherapy. Hawthorn contains a large number of phenolic compounds that have antioxidant and radioprotective effects on bone marrow cells in mice. The use of hawthorn extract in radiation-exposed personnel may also protect lymphocytes from radiation.[66,67] Additionally, hawthorn regulates immunity; its phenolic extract can regulate the lymphocyte subsets in mice and stimulate the humoral immune response in mice.[68] The expression of *TNF-α*, *IL-6*, and *IL-1B* is also significantly downregulated by hawthorn components, thereby mediating immunosuppressive effects.[69]

In the current study, data mining combined with a network pharmacology approach was used to determine the role of hawthorn in gastrointestinal tumors. However, the results predicted in this study have some limitations. First, studies on the ability of this plant to inhibit tumors are limited. Although the single herb was studied, there were many herbal components in the extract, the interactions between compounds were uncertain, and it was difficult to determine whether the correlation between compounds and their corresponding targets was direct or indirect, positive or negative. Therefore, it is necessary to integrate both the pharmacological and pharmacokinetic information of the chemical components to confirm the circulating concentration of the TCMs after oral administration to better understand the potential of Shan Zha in the treatment of gastrointestinal cancer. Second, network pharmacology is a research technique based on network data and computer simulation

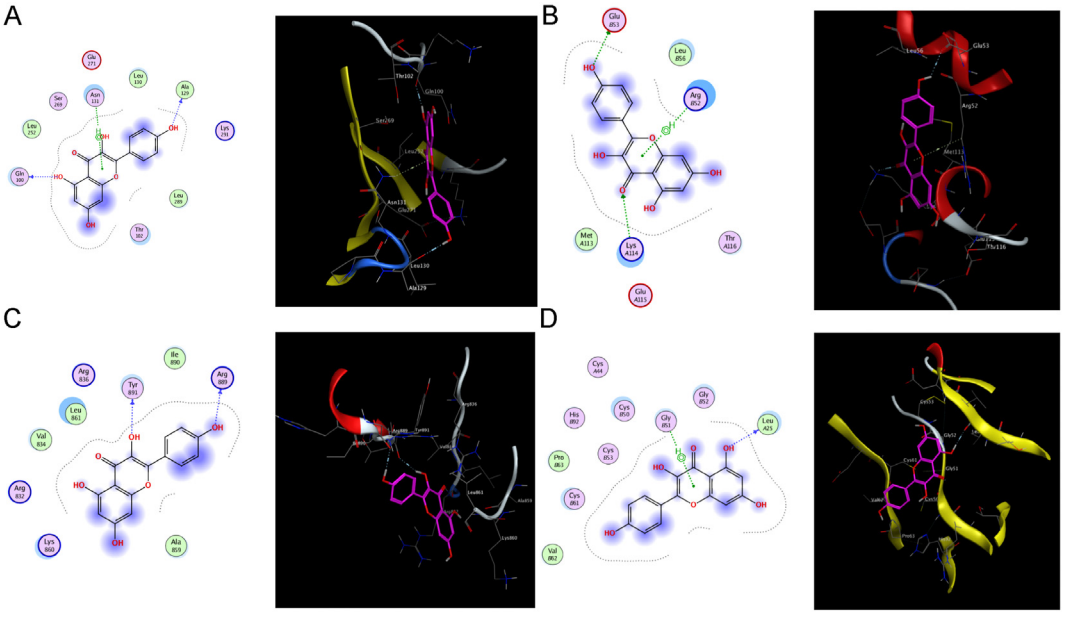


**Figure 7.** Diagram of molecular docking. The kaempferol and *TP53* (A), *CCND1* (B), *EGFR* (C), and *VEGFA* (D) molecular docking model diagrams. CCND1: Cyclin D1; EGFR: Epidermal growth factor receptor; TP53: Tumor protein p53; VEGFA: Vascular endothelial growth factor A.

analysis, the databases used are not updated promptly, and the accuracy and comprehensiveness of the target information need improvement. Therefore, the results obtained need to be validated using large sample sizes and multicenter experiments. Although the ability of hawthorn to affect lipid metabolism is recognized in this study, the molecular mechanism of this effect on gastrointestinal tumors, which is related to the regulation of the microenvironment surrounding tumor, has not yet been demonstrated. Therefore, *in vivo* or *in vitro* experiments are required to verify the results of this preliminary study.

In conclusion, this study predicted the main components and key targets of hawthorn extract in the treatment of gastrointestinal cancer through network pharmacology and explored its potential mechanism of action. Its main bioactive components, kaempferol, and quercetin, correspond to a variety of targets, potentially regulate BPs, such as proliferation, apoptosis, and oxidative stress, and have a potential inhibitory effect on gastrointestinal tumors. This study uses network pharmacology and molecular docking to lay a good theoretical foundation for the anticancer molecular mechanism of TCM, Shan Zha.

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## Author contributions

Wei Zhu and Ying Shen conceived the study; Jing Yang and Hong Zhou drafted the manuscript and performed the analyses; Jing Yang and Ling Cao performed the literature search and collected the data; Hong Zhou and Jialin Gu reviewed the manuscript and interpreted the data. All authors have read and approved the final manuscript.

## Ethics statement

No animals or humans were used in the studies that were the basis of this research.

## Data availability statement

The datasets presented in this study can be found in online repositories. Data used to support the findings of this study are available from the corresponding author upon request.

## Conflict of interest

None.

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