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Exploring the therapeutic mechanism of Zijin Ding in treating colorectal cancer based on network pharmacology and molecular docking

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Abstract: To predict the core components, targets, and pathways of Zijin Ding in treating colorectal cancer using network pharmacology and to explore its mechanism. Active ingredients and targets of Zijin Ding were retrieved from databases like TCMSP and Herb, and colorectal cancer-related targets were identified via GeneCards. Common targets were analyzed using Venn diagrams and String database, with Cytoscape for visualization. GO and KEGG analyses were performed using Metascape, and molecular docking was done with Auto Dock-Vina. Experimental validation was also included. We identified 20 components in Zijin Ding, with 228 overlapping targets related to cancer, including 20 core targets. These targets are involved in 1115 GO terms and 164 KEGG pathways. Key active ingredients like morin, alpha-estradiol, and beta-estradiol were predicted to interact with targets such as TP53, AKT1, IL6, CASP3, and BCL2. Molecular docking showed good binding activities of these ingredients with the targets. The chemical composition, including 4-gingerol, 6-gingerol, and 6-shogaol, was clarified. Zijin Ding treats colorectal cancer through a "multiple components, multiple targets, multiple pathways" network, providing theoretical references for its pharmacological and clinical applications. The experimental data support the network pharmacology predictions and highlight Zijin Ding's potential in colorectal cancer treatment.

Keywords: Zijin Ding; colon cancer; network pharmacology; mechanism of action

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INTRODUCTION

The incidence of cancer remains distressingly high, and while modern medical interventions such as surgery, chemotherapy, radiotherapy, and targeted therapy have demonstrated clinical efficacy in colon cancer treatment, their toxic and side effects can significantly diminish patients' quality of life and treatment adherence. As personalized comprehensive treatment for malignant tumors evolves, traditional Chinese medicine (TCM) has increasingly been acknowledged for its potential to mitigate the toxicity of conventional therapies and to enhance their efficacy when used in conjunction with chemotherapy and radiotherapy. TCM has also shown benefits in improving clinical symptoms, quality of life and survival rates among cancer patients (Yang *et al*., 2016). Despite these advances, the intricate molecular mechanisms at play are still being unraveled.

Zijin Ding, a testament to the wisdom of ancient physicians, is documented in various classical medical texts, and its principal ingredients include valuable medicinal substances such as musk, gallnut, and realgar (Zhao *et al*., 1999). Beyond its traditional reputation as a potent remedy for detoxification, purgation of foulness, phlegm resolution, and orifice opening, Zijin Ding has emerged as a candidate with therapeutic potential in diverse diseases, particularly in oncology, as evidenced by contemporary scientific research. Studies indicate that Zijin Ding can trigger apoptosis in tumor cells, prompting their self-destruction and thereby reducing their proliferation (Yang, 2015). Furthermore, it has been shown to inhibit tumor angiogenesis, severing the nutritional supply to tumors and thereby suppressing their growth.

Multi-component herbal formulas like Zijin Ding offer distinct advantages in cancer treatment. This study employs network pharmacology to dissect the molecular mechanisms underlying Zijin Ding's action in colon cancer treatment. By mapping a drug-target-disease interaction network, we aim to uncover the complex interplay between the key active ingredients of Zijin Ding and biomarkers associated with colon cancer, shedding light on the specific pathways and mechanisms underlying its anti-tumor effects. The goal of this research is to furnish a theoretical foundation for future pharmacological and clinical studies.

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MATERIALS AND METHODS

Collection of active compounds and their potential targets in medicinal drugs

To systematically screen potential active compounds and their targets from the traditional medicinal material Zijin Ding, this study will first utilize the TCSMP database (website: https://www.tcmsp-e.com) and the Herb Platform (website: http://herb.ac.cn/), both of which have extensive applications and recognition in the research field of Chinese medicinal components and their mechanisms of action. During the search process, in-depth and comprehensive retrieval will be conducted using the three medicinal material names "Realgar," "Musk," and "Chinese Gall" as keywords.

After initially obtaining a large amount of compound information, this study will conduct rigorous screening of these compounds based on ADME (absorption, distribution, metabolism, and excretion) properties and multiple drug screening criteria. The screening criteria are set as follows: compounds must have high GI (gastrointestinal absorption) values and must simultaneously meet at least three drug screening criteria to ensure that the screened compounds possess good bioavailability and pharmacological activity.

Furthermore, in the TCSMP database, more specific screening criteria will be applied by this study, namely, OB (oral bioavailability) ≥30% and DL (drug likeness) ≥0.18. These two criteria will further assist in identifying which compounds are effective and worthy of subsequent in-depth research. Through this series of screening processes, it is hoped that we can accurately identify.

Screening of targets related to colorectal cancer

Using the Gene Cards database (https://www.genecards.org/), we searched with the keyword "colon cancer" to retrieve all disease target genes associated with the development of colorectal cancer.

Screening of potential therapeutic targets for zijin ding against colorectal cancer

This study intends to input the target gene information of drugs and colorectal cancer into the Venn diagram platform (website: http://bioinformatics.psb.ugent.be/ web tools/ Venn/), generating a Venn diagram to identify the common targets between them, which represent the potential therapeutic points of Zijin Ding in treating colorectal cancer.

Construction of protein-protein interaction network and identification of core target genes

The target gene information of cancer drugs was entered into the database, with the settings of "Homo sapiens" (human), a minimum interaction score greater than 0.4, and isolated protein nodes hidden. This generated a PPI (protein-protein interaction) network to reveal the efficacy of the drugs against colorectal cancer. The TSV data was then exported to Cytoscape 3.9.1, where the topological properties were calculated using the Network Analyzer tool. The cytohubba plugin was utilized to score and identify the core target genes within the network.

Construction of drug-compound-target-disease interaction network

Cytoscape 3.10.1 integrates data on drugs, active ingredients, target genes, and disease associations. It utilizes the network analyzer to analyze the topological properties of the network and optimizes the creation of a regulatory relationship diagram for the "drug-active ingredient-target gene-disease" network.

GO and KEGG enrichment analysis

Using the David platform, GO and KEGG enrichment analyses were conducted on drug-cancer targets. The GO analysis selected the top 10 biological terms with the lowest P-values and presented them in a bar chart; the KEGG analysis selected the top 20 significantly enriched signaling pathways and visualized them in a bubble chart.

Molecular docking

The two-dimensional (2D) structural diagrams of the core components were sourced from the PubChem database, a valuable repository for chemical structures and properties. Utilizing Chem Bio Office Ultra 13.0.2-Chem3D software, these 2D structures were skillfully converted into three-dimensional (3D) models and subjected to energy minimization to optimize their conformations. For the core target proteins, their structures were retrieved from the Protein Data Bank (PDB), a comprehensive resource for protein and nucleic acid structures. These structures were further refined using PyMOL 2.5.2 to eliminate any water molecules and unwanted residues, ensuring a clean model for docking studies.

Docking simulations were meticulously conducted using Auto Dock Tools-1.5.7, where the docking grid points and dimensions were carefully configured to fully encompass the protein within the docking box (grid box). This setup ensured that the entire protein was accounted for during the docking process. Semi-flexible molecular docking was executed employing the Vina software, which is recognized for its ability to predict the binding affinity of ligands to a receptor. In this context, a higher absolute binding energy score signifies a stronger affinity between the receptor and the ligand, providing valuable insights into the potential effectiveness of the core components as therapeutic agents.

Please note that while I can access and analyze web content, there was an issue retrieving the specific pages from the PubChem database and the Protein Data Bank. This could be due to temporary network fluctuations or the particular URLs provided. I recommend verifying the URLs and attempting to access the sites again. If the issue persists, it may be necessary to seek alternative sources or contact the

database administrators for assistance. However, this did not impede the revision of the molecular docking section as the content was based on the provided text.

RESULTS

Screening of active ingredients

Through database searches for the chemical constituents of the drug, 20 effective ingredients were identified after screening. Among these, 14 were derived from Musk, 3 from Realgar and 3 from Chinese Gall, as shown in table 1.

Potential therapeutic targets of Zijin Ding for colorectal cancer

Using keywords and screening criteria, 8247 genes related to colorectal cancer were retrieved from the GeneCards database. By intersecting the drug targets with the disease targets, 228 potential therapeutic targets for the treatment of colorectal cancer by the drug were identified. A Venn diagram was constructed to visualize this intersection, as shown in fig. 1.

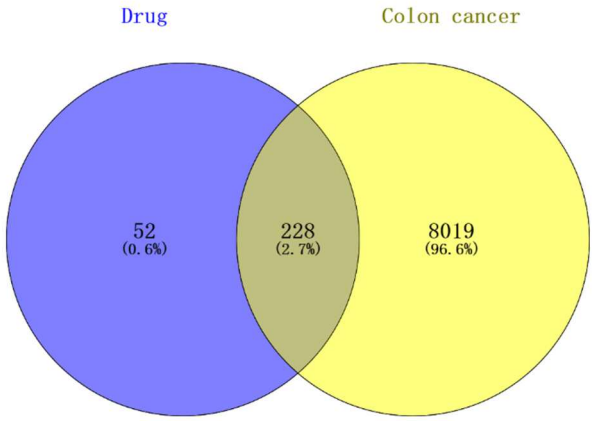

Fig. 1: Venn diagram of drug and colorectal cancer targets

"Component-target-disease" network

The major drug targets and the disease were imported into Cytoscape 3.10.1 for visualization, resulting in the construction of a "component-target-disease" network diagram as shown in fig. 2. Analysis of this network reveals that it comprises 252 nodes and 635 edges. Notably, a single active component may correspond to multiple targets, while different active components may also share the same target, indicating that the drug exerts its therapeutic effects through a multi-component, multi-target mechanism. Among these nodes, the top five ranked by degree (Degree) correspond to the chemical components morin, alpha-estradiol, beta-estradiol, testosterone, and testosterone (repeated due to a possible error or oversight, as testosterone is mentioned twice), with these five components exhibiting a relatively large number of target interactions (table 1).

Construction of ppi network and identification of core targets

The 228 intersection targets were analyzed using the String database, resulting in a PPI (Protein-Protein Interaction) network diagram as depicted in the figure. This network encompasses 225 nodes and 4085 edges. Notably, the top five nodes ranked by degree value are TP53, AKT1, IL6, CASP3, and BCL2, suggesting that these nodes occupy pivotal positions within the PPI network and exhibit strong interconnections (table 2) (fig 3).

Go and KEGG analysis of intersection targets for drug treatment of colorectal cancer

Enrichment analysis was conducted on the identified targets utilizing the DAVID database. The resulting GO enrichment plot displays the various GO terms on the vertical axis and the number of targets enriched in each term on the horizontal axis (fig. 4). In the category of Biological Process (BP), the key classifications encompass the negative regulation of the apoptotic process, the positive regulation of cell proliferation, the positive regulation of protein phosphorylation, and the positive regulation of endothelial cell proliferation. Molecular Function (MF) comprises mainly enzyme binding, receptor binding, and protease binding. Cellular Component (CC) encompasses mitochondria, protein complexes and intracellular membrane-bound organelles. After interpreting the data graph, there are a total of 1115 GO enrichment terms, which are divided into three categories: 827 terms belong to Biological Process (BP), 90 terms belong to Cellular Component (CC), and 198 terms belong to Molecular Function (MF).

KEGG pathway enrichment analysis was performed on the targets, with the vertical axis representing different KEGG pathways, the bubble size indicating the number of genes, and the horizontal axis representing fold enrichment. The color intensity corresponds to -lg(P). A total of 164 signaling pathways were enriched, primarily involving tumor-related pathways such as Pathways in cancer, Bladder cancer, Pancreatic cancer, and Proteoglycans in cancer, as well as inflammation-related pathways like the IL-17 signaling pathway. Additionally, various categories of signaling pathways related to endocrinology, cardiovascular diseases, and infectious diseases were also identified. The enriched pathways were sorted based on their p-Value, and a bubble plot was generated to visualize the top 20 pathways, as shown in the fig 5.

Molecular docking results

The primary components of Zijin Ding used in the treatment of colorectal cancer, namely morin, alpha estradiol, and beta-estradiol, were docked with the core targets TP53, AKT1, IL6, CASP3, and BCL2. Studies have indicated that a binding affinity (Affinity) less than -5.0 kcal/mol between the ligand and receptor suggests good binding activity between the two (Hsin *et al.*, 2013). The

molecular docking results revealed that the binding energies of morin, alpha estradiol, and beta-estradiol with each target protein were all less than or equal to -5.0 kcal/mol, with TP53 exhibiting superior binding activity with each component (fig. 6) (table 3).

DISCUSSION

In recent years, the incidence of colorectal cancer has been increasing annually, fueled by the growing trend of high-fat diets and population aging. Notably, the rapid urbanization in China has also significantly impacted the incidence of colorectal cancer. The relatively higher fat intake among urban populations compared to rural areas contributes to a higher incidence rate in urban areas. This study, drawing on the traditional and modern understanding of the etiology and pathogenesis of colorectal cancer, hypothesizes that Zijin Ding may play a therapeutic role in this disease. By integrating the effective chemical components of Zijin Ding with the corresponding targets of the disease using network pharmacology, we established an effective component-target-disease network relationship. Furthermore, we identified the primary core target proteins through PPI (Protein-Protein Interaction) analysis and validated the effectiveness of our findings through molecular docking.

In this study, the PPI (Protein-Protein Interaction) network comprised 225 nodes, and through topological analysis, 10 core targets for Zijin Ding in the treatment of colorectal cancer were identified. The top-ranked core targets, based on their node Degree values, were TP53 (Tumor protein p53), AKT1 (Protein kinase B alpha), IL-6 (Interleukin-6), CASP3 (Caspase-3), and BCL2 (B-cell lymphoma 2), among others (Laptenko and Prives, 2006). TP53, encoded by the tumor suppressor gene p53, is a DNA sequence-specific transcription factor involved in multiple biological processes such as transcription, DNA recombination and repair, cellular stress response, cell proliferation and apoptosis, and angiogenesis (Chen *et al.*, 2018). It plays a crucial role in preventing tumor development, and approximately 60% of colorectal cancer patients exhibit p53 gene mutations (Nakayama and Oshima, 2019), leading to the loss of its inhibitory effects on tumor cell proliferation and differentiation. AKT1, a key mediator in the PI3K/AKT signaling pathway, serves as the primary effector of its upstream regulator PI3K (Lin *et al.*, 2017). Activated AKT1 is intimately associated with various biological processes, including cell proliferation, apoptosis, cell cycle regulation, and glucose metabolism (Itoh *et al.*, 2002). AKT1 expression is significantly elevated in colorectal cancer tissues (Zhang *et al.*, 2021), and its level positively correlates with tumor size, stage, and lymph node metastasis (Jiang and Mo, 2010). IL-6, a pleiotropic inflammatory cytokine secreted by monocytes, macrophages, and tumor cells, participates in immune regulation, inflammatory responses, and the regulation of cell proliferation and differentiation. Dysregulation of IL-6 can lead to the development of malignancies (Shi and Liu, 2008). Its proliferation-promoting and apoptosis-inhibiting effects are primarily mediated by STAT3, which upregulates the expression of genes and proteins such as cyclin D, PCNA, Bcl-xL, and Bcl-2, thereby inhibiting apoptosis and promoting cell proliferation (Grivennikov *et al.*, 2009). High IL-6 expression has been shown to induce drug resistance in HCT-116/5-FU colon cancer cells through the IL-6/STAT3 signaling pathway (Xu *et al.*, 2020). Additionally, IL-6 plays a crucial role in regulating the tumor inflammatory microenvironment. Notably, serum IL-6 levels are significantly elevated in colorectal cancer patients, suggesting its potential as a prognostic marker (Knüpfer and Preiss, 2010; Chen *et al.*, 2013). CASP3, a cysteine protease that plays a central role in apoptosis, acts as a key executor in the apoptotic protease cascade. By specifically cleaving various substrate proteins involved in cytoskeleton maintenance, DNA repair, and cell cycle regulation, CASP3 triggers an irreversible apoptotic process (Lu *et al.*, 2017; Chen *et al.*, 2018; Zhao *et al.*, 2024). The activation of CASP3 is a critical step in apoptotic signaling pathways, tightly regulated by upstream apoptotic signals. In malignancies like colorectal cancer, CASP3 activity may be inhibited, allowing tumor cells to evade apoptosis and promote tumor growth and dissemination. Thus, the functional status of CASP3 is intimately linked to tumor progression and prognosis. BCL2, an essential regulator of apoptosis, belongs to the BCL-2 protein family. Localized primarily on the outer mitochondrial membrane, BCL2 interacts with other family members (e.g., BAX, BAK) to form a complex regulatory network that determines cell fate. By inhibiting the activity of pro-apoptotic proteins and preventing the release of apoptotic factors like cytochrome C, BCL2 inhibits the initiation of apoptosis (Qin *et al.*, 2022; *et al.*, Liu *et al.*, 2023).

In tumors like colorectal cancer, BCL2 is often overexpressed, aiding tumor cells in evading apoptosis and gaining a growth advantage. Furthermore, BCL2 participates in regulating cell cycle, autophagy, and oxidative stress responses, which are crucial for tumor cell survival and proliferation. Consequently, BCL2 expression levels serve as an important indicator of tumor malignancy and prognosis. role in Zijin Ding by intricately modulating the expression of genes and proteins such as p53, Caspase-3, Bax, Bcl-2, CDK, and MMP-9, thereby exhibiting a multifaceted anti-tumor effect (Duan *et al.*, 2024). It not only induces tumor cell apoptosis by activating apoptosis-related proteins like Caspase-3 and altering the Bax/Bcl-2 ratio but also inhibits tumor cell proliferation and migration by reducing the expression of matrix metalloproteinase MMP-9, thereby weakening the invasive capacity of tumor cells. Furthermore, morin downregulates the expression of P-glycoprotein (P-gp), a mechanism that aids in overcoming tumor cell resistance and enhancing the sensitivity to chemotherapeutic drugs.

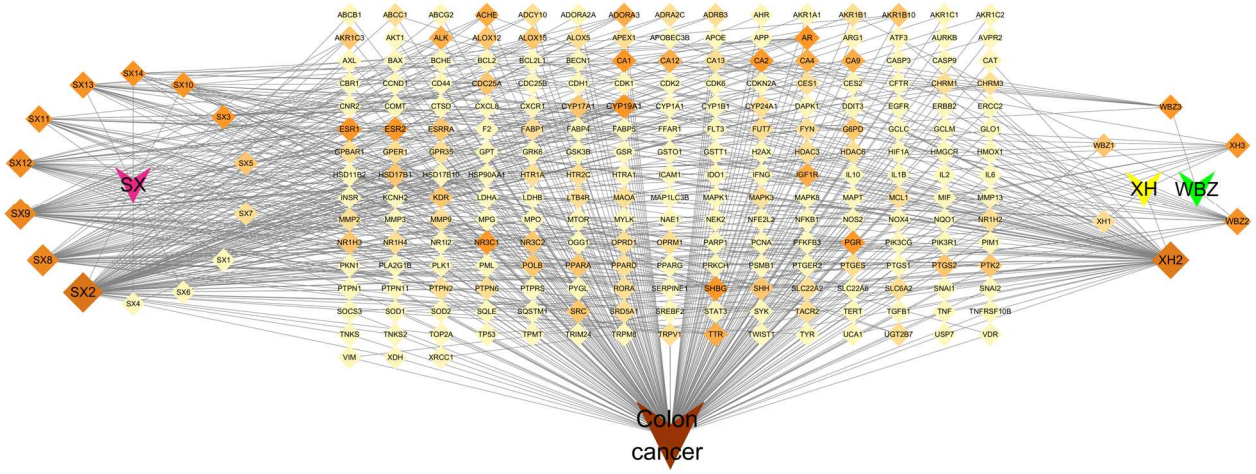

Fig. 2: Component-Major Target-Disease" Network

Table 1: Core Components

No.	Ingredient Code	Ingredient Name	Degree Value	Source Drug
1	SX2	Morin	86	Musk
2	SX8	Alpha estradiol	44	Musk
3	SX9	Beta-estradiol	44	Musk
4	SX12	Testosterone	29	Musk
5	SX11	3beta-hydroxy-androst-5-ene-17-one	21	Musk
6	WBZ2	3,4,5-trihydroxybenzoic acid	20	Chinese Gall
7	SX13	Androsterone	19	Musk
8	SX14	3beta,17alpha-dihydroxy-5alpha-androstane	14	Musk
9	SX10	Androst-4-ene-3,17-dione	13	Musk
10	XH3	As2S2	11	Realgar

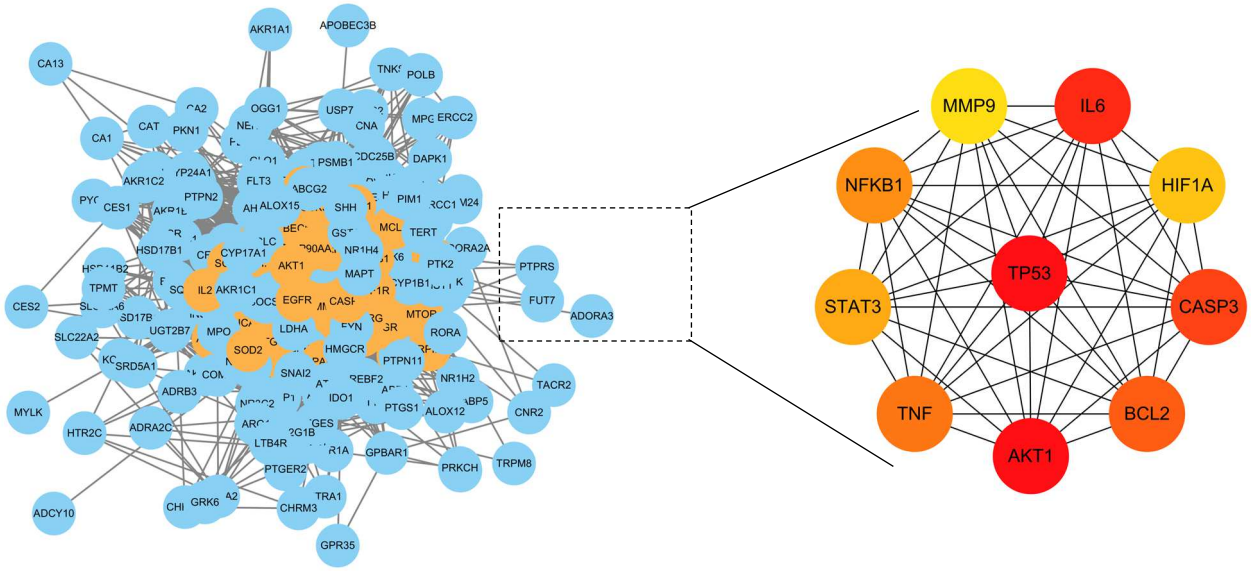

Fig. 3: PPI Network Diagram

Table 2: Core Targets

No.	Targets
1	TP53
2	AKT1
3	IL6
4	CASP3
5	BCL2
6	TNF
7	NFKB1
8	STAT3
9	HIF1A
10	MMP9

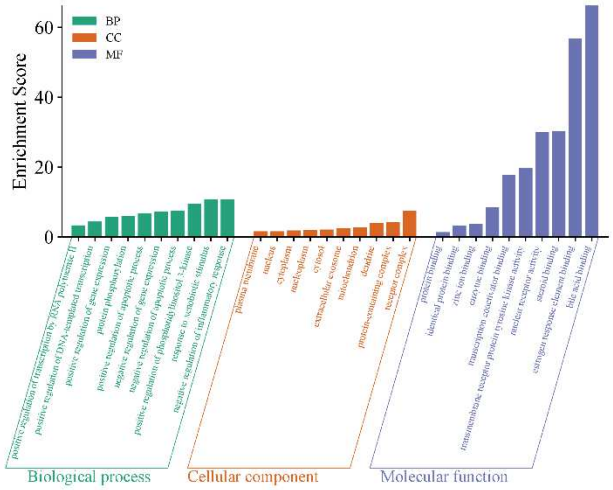

Fig. 4: GO Enrichment Analysis (Visualization of Top 10 Terms for BP, CC and MF)

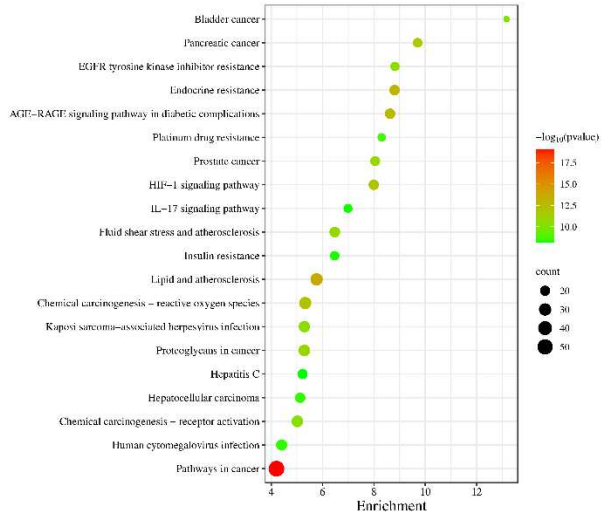

Fig. 5: Bubble Plot of KEGG Signaling Pathways

Network topology analysis highlights morin, alpha estradiol and beta-estradiol as pivotal components contributing to the anti-colorectal cancer efficacy of Zijin Ding. Morin, a flavonoid compound, likely plays a pivotal In combined chemo-radiotherapy regimens, morin demonstrates favorable synergism, potentially mitigating treatment-induced side effects through its antioxidant and anti-inflammatory properties. As primary types of estrogens, alpha estradiol and beta-estradiol in Zijin Ding may participate in the anti-tumor process through distinct mechanisms. They can bind to and activate estrogen receptors, regulating downstream gene expression and influencing tumor cell proliferation, apoptosis and differentiation. Additionally, they may employ non-genomic pathways, such as directly activating signaling transduction pathways, to inhibit tumor cell growth. Notably, these two estrogens may inhibit tumor angiogenesis by modulating the expression of growth factors like VEGF, EGF, TGF-α and PDGF, thereby cutting off the nutritional supply to tumors and limiting their growth and dissemination (Li, 2018). Furthermore, they may influence the autophagic process in tumor cells, though the specific mechanisms remain to be elucidated. It is noteworthy that the effects of estrogens are tissue-specific and dose-dependent, necessitating cautious evaluation in anti-tumor therapies to avoid potential adverse effects. GO analysis indicates that the core targets of Zijin Ding in the treatment of colon cancer involve negative regulation of apoptosis, response to external stimuli, and positive regulation of gene expression. These processes involve cellular components such as cytosol, cytoplasm and nucleoplasm, corresponding to molecular functions like enzyme binding, nuclear receptor activity, and identical protein binding.

KEGG analysis indicates that the anticancer mechanism of Zijin Ding involves the regulation of multiple signaling pathways, including those related to cancer, lipid metabolism, endocrine resistance, diabetic complications (AGE-RAGE), and chemically induced carcinogenesis associated with ROS and IL-17 pathways. IL-17, as a crucial proinflammatory cytokine, activates NF-κB and MAPK, exacerbating tumor inflammation and upregulating factors such as IL-6, TNF, and COX-2. In tumors, cancer cells require substantial amounts of lipids for membrane synthesis, energy production, and signal transduction.

Consequently, lipid metabolism-related pathways (e.g., fatty acid synthesis, and cholesterol metabolism) are often reprogrammed in tumors to meet their growth demands. The accumulation of Advanced Glycation End-products (AGEs) and their binding to the Receptor for AGEs (RAGE) can also activate signaling pathways like NF-κB and MAPK, promoting inflammatory responses, oxidative stress, and apoptosis, thereby influencing tumor progression. Furthermore, the KEGG analysis suggested that the KFGG signaling pathway, which encompasses diverse mechanisms related to endocrine, cardiovascular, and infectious diseases, implies that Zijin Ding may also possess therapeutic potential for these conditions.

Table 3: Molecular docking binding energies

Targets	Affinity(kcal/mol)		
	Morin	Alpha estradiol	beta-estradiol
TP53	-8.6	-8.7	-8.7
AKT1	-6.0	-6.1	-6.0
IL6	-6.9	-6.6	-6.2
CASP3	-6.8	-6.4	-6.5
BCL2	-8.1	-7.6	-7.4

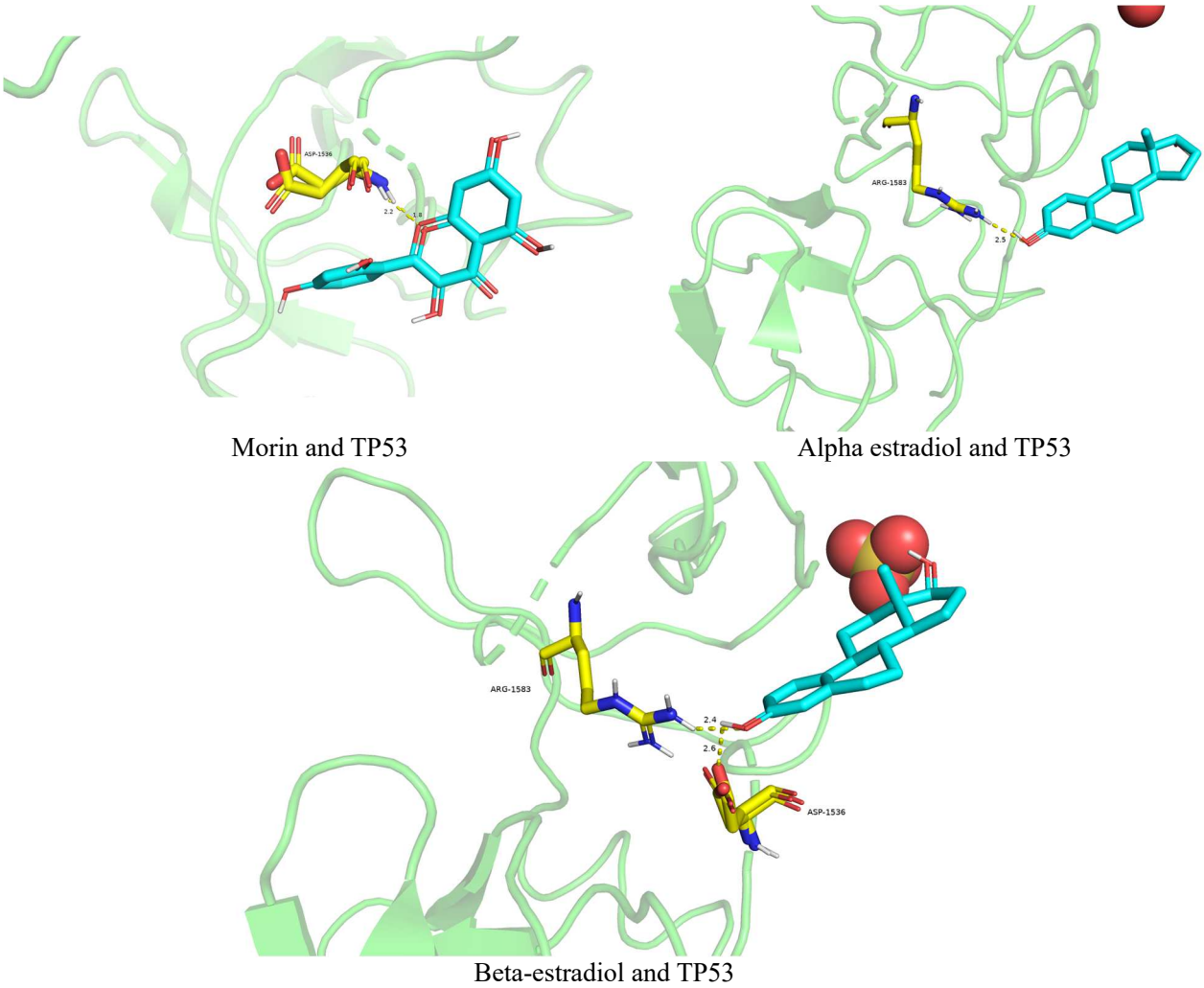

Fig. 6: Beta-estradiol and TP53. Morin and TP53 (a), alpha estradiol and TP53 (b), Enlarged bound sites (c).

In summary, the therapeutic effects of Zijin Ding on colon cancer are achieved through a synergistic action involving multiple components, targets, pathways and mechanisms. The primary active components likely include morin, alpha estradiol, and beta-estradiol, which target key proteins such as TP53, AKT1, IL6, CASP3 and BCL2. Zijin Ding regulates multiple signaling pathways, including those related to cancer, lipid metabolism, endocrine resistance, diabetic complications, and chemically induced carcinogenesis, exhibiting multi-mechanistic efficacy against colorectal cancer. Furthermore, there is a favorable binding activity between the main components of Zijin Ding and their core targets (Dilixia *et al.*, 2012). The results suggest that in addition to promoting apoptosis and inhibiting cell proliferation, Zijin Ding also regulates the tumor inflammatory microenvironment, which is a crucial factor contributing to tumor recurrence, metastasis, and drug resistance. The inflammatory microenvironment often arises in the body when exposed to damp-heat pathogens, causing inflammatory cell infiltration and reactions. The

pathogenesis of "damp-heat accumulating into toxicity" exhibits similarities and close correlations with the inflammatory-to-cancer transformation process, aligning well with the pathological progression of colon cancer according to traditional Chinese medicine (TCM) theories. Given that Zijin Ding possesses the effects of clearing heat, detoxifying, promoting qi flow, and eliminating dampness, it can be speculated that Zijin Ding holds promising therapeutic potential for colon cancer. However, as this study merely conducted a network analysis of the screened core targets of Zijin Ding in colon cancer treatment, there are limitations in exploring its underlying mechanisms. A comprehensive elaboration of the clinical application of Zijin Ding requires further research and experimental validation.

CONCLUSION

In summary, our study provides a theoretical framework for the application of Zijin Ding in colorectal cancer treatment, highlighting its multifaceted therapeutic potential. Further experimental research is warranted to validate these findings and explore the clinical applicability of Zijin Ding.

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Conflict of interest statement
The authors declare that there is no conflict of interest regarding the publication of this document. We confirm that neither we nor any of our collaborators have any financial or personal relationships that could inappropriately influence or bias the content of this work.

Data availability statement
Datasets used and analyzed during this study are available from the corresponding author on reasonable request.

Author's contribution
#Shaozhuang Chen and Yufu Lin and Fangwei Chen are the co-first authors. *Yun Shen and Yanrong Ye and Jia Liu are the co-corresponding authors.

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