Research article

Mechanism of Dahuang Mudan Decotion in the treatment of colorectal cancer based on network pharmacology and experimental validation

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ABSTRACT

Objective: The objective of this study was to assess the pharmacological activity and therapeutic mechanism of Dahuang Mudan Decotion (DHMDD) for colorectal cancer using ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS), network pharmacology and in vitro experiments.
Methods: The chemical components of DHMDD were identified by UPLC-MS. Network pharmacological analysis was utilized to screen the active ingredients and targets associated with DHMDD for colorectal cancer. Based on the results of network pharmacology, the potential mechanism of DHMDD on colorectal cancer predicted was experimentally studied and verified in vitro.
Results: DHMDD primarily exerts its effects on colorectal cancer through 52 active ingredients. AKT1, ESR1, HSP90AA1, JUN, PIK3CA, PIK3CB, PIK3R1, SRC, STAT3, TP53 were the top 10 targets. The top 10 ingredient nodes were Quercetin, Physcione, Pontigenin, Crysophanol, Linolenic acid, Piceatannol, Adenosine, Emodin, Sambunigrin, and Prunasin. The main compounds and the target proteins exhibited strong binding ability in molecular docking studies. The results of cell experiments demonstrated that DHMDD can inhibit the proliferation, invasion and migration of CRC cells through the PI3K/Akt pathway.
Conclusion: Through network pharmacology analysis and cell experiments, this study suggests that DHMDD can exert its therapeutic effects on colorectal cancer through a combination of multiple components and targets.

1. Introduction

Colorectal cancer (CRC) is a common malignancy of the digestive tract that poses a significant health risk. The 2020 Cancer Statistics report published by the International Agency for Research on Cancer (IARC) revealed that CRC is the third most frequently diagnosed cancer and the second leading cause of cancer-related mortality worldwide. In 2023, approximately 153,020 individuals

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were diagnosed with colorectal cancer. China has higher incidence and mortality rates of CRC compared to the global average [1,2]. In recent years, dietary and lifestyle changes have occurred alongside socio-economic development and improvements in living standards. There is a distinct trend of colorectal cancer patients getting younger, as the number of individuals under 50 years old continues to rise annually [2]. CRC is characterized by a subtle onset, challenging early detection, and a bleak prognosis, resulting in many patients missing the opportunity for optimal surgical intervention. By the time typical symptoms manifest, the tumor is already in the intermediate or advanced stages, with a five-year survival rate of less than 50 % [3]. Therapeutic strategies for CRC encompass surgery, chemotherapy, radiation therapy, and immunotherapy. However, the clinical efficacy and prognosis of these approaches are unsatisfactory, often accompanied by severe side effects and high toxicity [4]. Traditional Chinese Medicine (TCM) is extensively utilized as an adjunct therapy for CRC in China. Additionally, TCM has been found to impact cancer prognosis. Clinical studies have demonstrated the significant role of TCM in ameliorating patients' clinical symptoms, boosting immunity, reversing chemotherapy resistance, and preventing tumor recurrence and metastasis [5]. Therefore, there is promising potential in exploring therapeutic drugs for CRC derived from TCM that exhibit notable efficacy with minimal side effects.

Dahuang Mudan Decotion (DHMDD) is a renowned traditional Chinese herbal prescription that was originally documented in ShanghanLun, a well-known text on TCM authored by Zhang Zhongjing [6]. It is composed of five TCMs, including *Rheum potaninii* Losinskaja (Dahuang in Chinese), *Moutan Cortex* (Moudanpi in Chinese), *Prunus persica* L. *Batsch.* (Taoren in Chinese), *Semen Benincasae (Dongguazi in Chinese)* and *Natrii Sulfas* (Mangxiao in Chinese). DHMDD had been found to have an anti-cancer effects for CRC related intestinal diseases [7–9]. DHMDD mitigates ulcerative colitis in mice by modulating ILC3 function to repair intestinal barriers [10], and by adjusting gut microbiota to restore Th17/Treg balance [11]. The main pharmacological ingredients include emodin, rhein, aloe emodin, chrysophanol, emodin methyl ether, paeoniflorin, paeonol, amygdalin, prunasin etc. Emodin reduces tumor burden in colorectal cancer by decreasing M2-like macrophages [12]. Rhein enhances the sensitivity of human colorectal cancer cells to EGFR inhibitors by blocking the STAT3 pathway [13]. Aloe emodin inhibits EGF-induced neoplastic cell transformation by targeting the ERK/MSK1 and AKT/GSK3β signaling pathways [14]. Chrysophanol suppresses proliferation, invasion, and tumorigenesis of colon cancer SW480 cells in nude mice via the AMPK-dependent signaling pathway [15]. Emodin methyl ether effectively suppresses the release of inflammatory factors during intestinal epithelial cell injury, mitigating LPS-induced intestinal epithelial cell damage by boosting autophagy proteins like Beclin-1 and LC3 while inhibiting the expression of gap junction protein Cx43 [16]. Paeoniflorin combats colorectal cancer cell stemness through the miR-3194–5p/catenin beta-interacting protein 1 axis [17]. However, there is a limited number of scientific studies of DHMDD on the mechanisms of CRC treatment. There is also a lack of systematic and comprehensive analysis and identification of DHMDD chemical components. Therefore, the aim of this study was to characterize the chemical components of DHMDD and explore the role of DHMDD in colorectal cancer and its molecular mechanism. It provides theoretical basis for clinical application of DHMDD and elucidation of its pharmacological substance basis.

Network pharmacology has emerged as a valuable approach for comprehending the intricate interactions between molecules and proteins, offering significant potential in elucidating the pathogenesis and progression of diseases, TCM syndromes, and the mechanisms underlying TCM's therapeutic effects. The integration of network pharmacology and in vitro experiments represents an effective strategy for elucidating the anti-cancer effects of traditional Chinese medicine. These findings provide novel insights into the mechanism of action of DHMDD in CRC treatment and offer a scientific foundation for its clinical application.

2. Materials and methods

2.1. Materials and reagents

Formic acid was obtained from Tianjin Kermel Chemical Reagent Co., Ltd. in Tianjin, China. Methanol and Acetonitrile (HPLC grade) were supplied by Fisher Scientific Corporation in Pittsburgh, PA, USA. Chromatographic pure Leucineenkephalin was purchased from Sigma-Aldrich in St Louis, MO, USA. Ultra-pure water was produced using a Milli-Q Ultra-pure water system from Millipore in Billerica, USA. Other reagents and chemicals were of analytical grade. The crude drugs, including Dahuang, Mangxiao, Taoren, Mudanpi, and Dongguazi, were obtained from Harbin Tongrentang Drug Store in Harbin, China. This study was conducted at the Faculty of Pharmacy, Heilongjiang University of Chinese Medicine.

The CRC cell line HCT116 was purchased from the Cell Bank of *the* Chinese Academy of Sciences (Shanghai, China). Cells were cultured in MCCOY'S 5A medium (Solarbio, China) supplemented with Fetal bovine serum (CELL-BOX, China). Penicillin-streptomycin was obtained from. Beyotime Biotechnology (Shanghai, China). CCK-8 Kit from Seven biotech (Beijing, China)was used to mesure the cell Cytoactive. The 24-well Transwell insert system (Corning, NY, USA, aperture 8 mm) was used to measure the ability of cells to invade. Matrigel was purchased from Corning (NY, USA). Total RNA Extraction Kit from Seven biotech (Beijing, China)was used to extract total RNA from cells. Two Step RT and qPCR Kit from Seven biotech (Beijing, China) was used to detect mRNA level expression in cells.Primary antibody β-Actin, AKT1, p-AKT were purchased from Beyotime Biotechnology (Shanghai, China) and Bcl-2, BAX were purchased from Affinity Biosciences (Jiangsu, China). HRP-conjugated anti-rabbit IgG and anti-mouse IgG were purchased from Beyotime Biotechnology (Shanghai, China).

2.2. Preparation of Dahuang Mudan decoction

The DHMDD was prepared according to the method and procedure described in Shang-Han-Lun and was optimized: Dahuang (55.2 g), Mudanpi (13.8 g), Taoren (15 g), and Dongguazi (30 g) were soaked in double-distilled water for 30min at a ratio of 1:10 (m/v), decocted for 45 min, then added Mangxiao (48 g), decocted for 2min. The resulting filtrates were mixed, condensed by heating

evaporation, and dried using a vacuum freeze drier.

2.3. Component identification analysis of DHMDD decoction

A 500 mg DHMDD freeze-dried powder was weighed and then sufficiently dissolved in 20 ml 75 % methanol, the solution was sonicated for 30 min and centrifuged at 13000 rpm for 15 min at 4 °C, and then filtered through a 0.22um filter and analyzed by UPLC. Chromatographic separation of the metabolites was performed on the Acquity UPLC system (Waters Corporation, Milford, Massachusetts) by an Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm, Waters) at 35 °C column temperature.

Chromatographic conditions: Column temperature was 35 °C, injection volume was 4 μL, flow rate was 0.3 mL/min, Mobile phase: Mobile phase A consisted of water and 0.1 % formic acid; mobile phase B consisted of methanol with 0.1 % formic acid with the gradient elution mode (0–1.5 min, 99-95 % A; 1.5–12 min, 95–85 % A; 12–20min, 85-64%A; 20–22min, 64-50%A; 22–25min, 50-1% A, 25–27min, 1%A; 27–28 min, 1–99%A; 28–30min, 99%A).

Mass spectrometry conditions: Capillary voltage was 3.0 kV in ESI + mode and 2.8 kV in ESI- mode, sampling cone voltage was 30 V, desolvation temperature was 350 °C, desolvation gas flow was 600L/h. The Q-TOF Premier acquisition rate was fixed to 0.4 s/scan, with 0.1s inter scan delay. The mass range was set at m/z 50–1500 using extended dynamic range. Leucine-enkephalin was used for mass accuracy correction during the whole experiment.

2.4. Network pharmacology

2.4.1. Establishment of component target database for DHMDD

The constituents identified from DHMDD were entered into the PubChem database [18](https://www.ncbi.nlm.nih.gov/pubmed/) to obtain the SMILES and SDF files. Subsequently, these files were input into the Swiss ADME database (http://www.swissadme. ch/) [19] for further analysis. The active constituents were then screened using Lipinski's rule of five [20], the selection criteria were as follows: (1) Molecular weight <500, (2) Rotatable bonds ≤10, (3) H-bond acceptors ≤10, (4) H-bond donors ≤5, (5) Log Po/w ≤ 5. If a compound violates more than two of the five rules, it should be further investigated by conducting a literature search to identify the main active components that have been previously reported. Then, the component targets were found by inputting them into the Swiss Target Prediction Database (http://www.swisstargetprediction.ch/). targets <0.5 probability were removed. In order to gather more information about the targets, the names of the ingredients were entered into the Traditional Chinese Medicine Systems Pharmacy Database and Analysis Platform (TCMSP) [21].

2.4.2. Establishment of therapeutic target database for CRC

The information on therapeutic target was searched by using "colorectal cancer" as the keyword. The therapeutic targets database can be obtained from OMIM [22], GeneCards [23], TCMSP, and TTD [24] databases.

2.4.3. Enrichment analysis and construction of the components-targets-pathway network

We employed the cluster profile R package to analyze the potential role of active components in DHMDD in gene function and signaling pathways, specifically focusing on the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. GO terms and KEGG pathways were deemed statistically significant if they had a p-value <0.05. We constructed a components-targets network for DHMDD in the treatment of CRC using the network-visualization software Cytoscape v3.8.0.

2.4.4. Molecular docking study of target proteins and active components of DHMDD

Suitable target protein structures were selected from the PDB database [25], with the species defined as "*Homo sapiens*," fine resolution less than 2.5, and small molecular compounds attached as reference standards. Obtain the SDF file of the 3D structure of the active ingredients of DHMDD from the PubChem database. AutoDock software [26] was utilized to dehydrate the target protein, adjust the size and position of the docking box, and optimize the conformation of the ligand and receptor. The results were outputted in the "pdbqt" format. PyMol 2.4.0 [27]was employed to visualize the results of molecular docking, and the docking affinity, hydrogen bonding, and bond length reflected the stability of intermolecular interactions.

2.5. Experimental validation in vitro

2.5.1. CCK8 assay

Colorectal cancer HCT116 cells in the logarithmic growth phase were harvested, and the cell density was adjusted to 5×10^3 cells/ mL. Subsequently, 100 μL of cell suspension was seeded into each well of a 96-well plate. DHMDD was dissolved in fresh culture medium and configured with different concentrations. Following a 24-h incubation at 37 °C with 5 % CO2, the culture medium was aspirated, and 100 μL of fresh culture medium containing various concentrations of DHMDD (0, 1, 2, 4, 8, 16, 32, 64 mg/ml) was added to each well. Incubate the plate in a 37 °C incubator with 5 % CO2 for 24 h. Add 10 μL of CCK-8 reagent to each well, mix thoroughly, and incubate for 60 min in the incubator. Measure the absorbance at a wavelength of 490 nm using a microplate reader and calculate the results. The inhibitory effect of DHMDD on the proliferation of colorectal cancer cells was evaluated, and a line graph was plotted.

2.5.2. Colony formation assay

The experiment was divided into a control group and an experimental group. The experimental group was treated with DHMDD for

24 h. Monolayer-cultured cells in the logarithmic growth stage were dissociated into single cells using 0.25 % trypsin, counted, and then suspended in culture medium containing 10 % fetal bovine serum. 700 cells were seeded per well in a six-well plate and cultivated for 14 days until colonies were visible to the naked eye. The number of cell clones formed in the 6-well plate was quantified using ImageJ software and the difference between the two groups was calculated.

2.5.3. Transwell assay

Cell invasion ability was assessed using the transwell assay. The Matrigel was taken out in advance and placed it in a 4 °C refrigerator to thaw for later use. The bottom of the upper side of the Transwell chamber was coated with Matrigel. Placed in the incubator for 3 h and taken out for use when a white layer appeared. Colorectal cancer HCT116 cells were divided into control group and DHMDD group and treated for 24 h. Then the HCT116 cells were adjusted to 2.0×10^5 cells/ml in the serum-free medium and culture medium containing 10 % FBS was added to the lower chamber and incubated for 48 h in a cell incubator. The Transwell chamber was taken out, and the culture medium was aspirated. It was washed twice with PBS, and then 4 % paraformaldehyde was added to fix it for 10–15 min. After that, it was stained with 0.1 % crystal violet for 5–10 min. The number of cells passing through the membrane were counted by an inverted microscope. ImageJ software was used for image analysis.

2.5.4. Wound healing assay

The cell migration ability was assessed using a wound-healing assay. Briefly, HCT116 cells were seeded in 6-well plates (1×10^6 cells/well). When reaching 100 % confluence, a scratch was generated using a 10-µl pipette tube. The debris was removed by washing with phosphate buffered saline (PBS), then incubated with serum-free medium and medium containing DHDDT for 24 h. Images were captured using a light microscope at 0 h and 24 h. ImageJ software was used for image analysis, then the migration rate was assessed.

2.5.5. Quantitative real-time polymerase chain reaction (qRT-PCR)

Based on the findings of network pharmacology, we chose AKT, PI3K, PI3KCA, and PI3KR1 to investigate the mechanism by which DHMDD promotes cell proliferation in colorectal cancer (CRC). Total RNA was isolated from the cultured cells using the SevenFast Total RNA Extraction Kit. Subsequently, reverse transcription was performed, and qRT-PCR was carried out using the SevenFast Two Step RT and qPCR Kit. β-actin as internal reference. Fluorescence signal was confirmed by melting curve analysis, and mRNA levels of target genes were calculated using the $2^{-\Delta\Delta CT}$ method. The primer sequences utilized for qRT-PCR are provided in Table 1.

2.5.6. Western blotting

Colorectal cancer HCT116 cells in the logarithmic growth phase were harvested and seeded in 6 cm culture dishes at a density of 3×10^5 cells per dish. After 24 h, the cells were divided into two groups: the Control group and the DHMDD group. Cells from each group were collected 24 h later. The cells were washed twice with $1 \times$ PBS, and total proteins were extracted using RIPA buffer. The protein concentrations were determined using a BCA protein assay kit. The protein samples were transferred onto PVDF membranes. Subsequently, the membranes were incubated overnight at 4 °C with primary antibodies targeting β-Actin, AKT1, p-AKT, Bcl-2, and BAX, and then incubated with HRP-conjugated secondary antibodies. The grayscale values were quantified using Image Lab software.

2.5.7. Statistical analysis

Data are presented as the mean ± SD from ≥3 independent experiments. Data were analyzed using one-way ANOVA among multiple groups. The experimental results were subjected to statistical analysis using GraphPad Prism 8.0 software. Differences in experimental data among groups were compared using single-factor analysis of variance (ANOVA) followed by Dunnett's test. A significance level of $\alpha = 0.05$ was used as the threshold for statistical significance.

3. Results

3.1. Identification of chemical compounds in DHMDD

The chemical constituents of DHMDD were analyzed using a rapid analysis technique combined with UPLC-QTOF-MS/MS (Fig. 1A

Table 1
Real-Time polymerase chain reaction primers.

Gene	Sequence (5′-3′)
AKT1-F	5′-AGAAGCAGGAGGAGGAGGAG-3′
AKT1-R	5′-CCCAGCAGCTTCAGGTACTC-3′
PI3K–F	5′-AAGCAGTGCCTGTAGGAGGA-3′
PI3K–R	5′-TGTCGATGAGCTTTGGTGAG-3′
PIK3CA-F	5′-CATGCATTGTTTTGCACCCC-3′
PIK3CA-R	5′-ATGGAAGACGGGAGATTCACAT-3′
PIK3R1-F	5′-TCTACCCAGTGTCCAAATACCAG-3′
PIK3R1-R	5′-TAAATGCTTCGATAGCCGGTTC-3′
β-actin-F	5′-CAGATGTGGATCAGCAAGCAGGA-3′
β-actin-R	5′-CGCAACTAAGTCATAGTCCGCCTA-3′

and B). Both positive and negative ionization modes were employed, and the analysis involved comparing the retention behavior, MS/MS data, and mass spectrometry characteristics of known reference compounds. A total of 81 ingredients in DHMDD were identified by matching them against a chemical library, as presented in Supplementary Table S1. Out of these, 35 compounds were identified as Dahuang, 3 as Dongguazi, 34 as Mudanpi, 7 as Tairen, and 2 as a combination of Dahuang and Mudanpi.

3.2. Targets and network analysis of DHMDD against CRC

A total of 65 active compounds underwent ADME screening (Fig. 2). The SMILES of the 65 active compounds were used to predict their potential targets by querying SwissTargetPrediction and TCMSP databases. After removing duplicates, a total of 562 genes related to the compounds were collected. Additionally, a database search retrieved a total of 1900 genes related to CRC (Supplementary Table S2). The analysis revealed 224 overlapping genes when comparing the 562 compound-related genes with the 1900 disease-related genes (Fig. 2A; Supplementary Table S2). A compound-target network of DHMDD against CRC was constructed using Cytoscape 3.8.0, incorporating the 65 active compounds and 224 overlapping genes (Fig. 2B). The network demonstrated the differential contributions of these active compounds and genes to DHMDD against CRC. The targets were uploaded to the String database, restricting the species to "*Homo sapiens*", and a confidence level of 0.900 was used to construct a PPI network graph (Fig. 3A). The protein interactions were imported into Cytoscape 3.8.0 software, and topological analysis was conducted using the NetworkAnalyzer tool (Fig. 3B). The top 10 targets based on the degree parameter were AKT1, ESR1, HSP90AA1, JUN, PIK3CA, PIK3CB, PIK3R1, SRC, STAT3, and TP53 (Fig. 3C). The top 10 ingredient nodes with the highest degree, betweenness centrality, and closeness centrality in the topological analysis were Quercetin, Physcione, Pontigenin, Crysophanol, Linolenic acid, Piceatannol, Adenosine, Emodin, Sambunigrin, and Prunasin, indicating their strong associations with multiple targets. GO and KEGG enrichment analyses were conducted on the aforementioned 224 targets to investigate their involvement in the treatment of CRC. Fig. 4A/B/C displays the top 20 terms in the GO biological process (BP), cellular component (CC), and molecular function (MF) ranked by p-value. The analysis revealed that these potential targets were primarily associated with the membrane raft, membrane microdomain, and membrane region, and they were involved in regulating molecular functions such as protein serine/threonine kinase activity, protein tyrosine kinase activity, phosphatase binding, oxidoreductase activity under oxidative stress, protein kinase B signaling, peptidyl-tyrosine phosphorylation, inflammatory response, and protein kinase B signaling. Furthermore, the results of the KEGG analysis showed significant enrichment of 224 overlapping genes between DHMDD and CRC in 20 signaling pathways (p < 0.05). These pathways include the PI3K-Akt signaling pathway, Lipid and atherosclerosis, Proteoglycans in cancer, Chemical carcinogenesis-receptor activation, Fluid shear stress and atherosclerosis, Endocrine resistance, AGE-RAGE signaling pathway in diabetic complications, HIF-1 signaling pathway, and EGFR tyrosine kinase inhibitor resistance (Fig. 4D). Results were visualized with a heatmap (Fig. 5A), revealing that DHMDD's core target and main active ingredients exhibit good binding ability, suggesting a synergistic effect on the core target through multi-component action. High scores in DHMDD (Physcione, Crysophanol, Quercetin, and Emodin) and their targets were visualized using PyMol 2.4.0 (Fig. 5B).
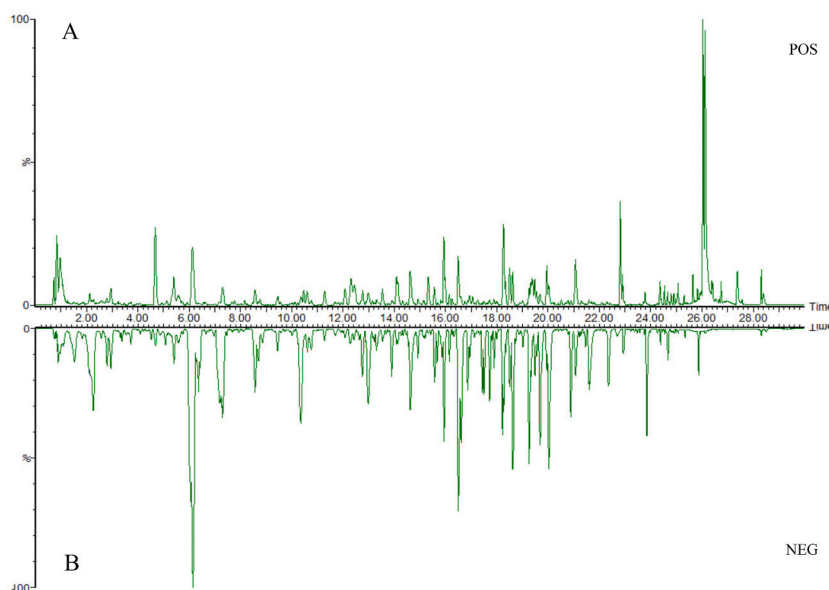

Fig. 1. Representative base peak intensity (BPI) chromatograms of DHMDD in (A) positive and (B) negative modes by UPLC-ESI-Q-TOF/MS. The numbering of identified compounds (1−81) is the same as in Supplementary Table S1.
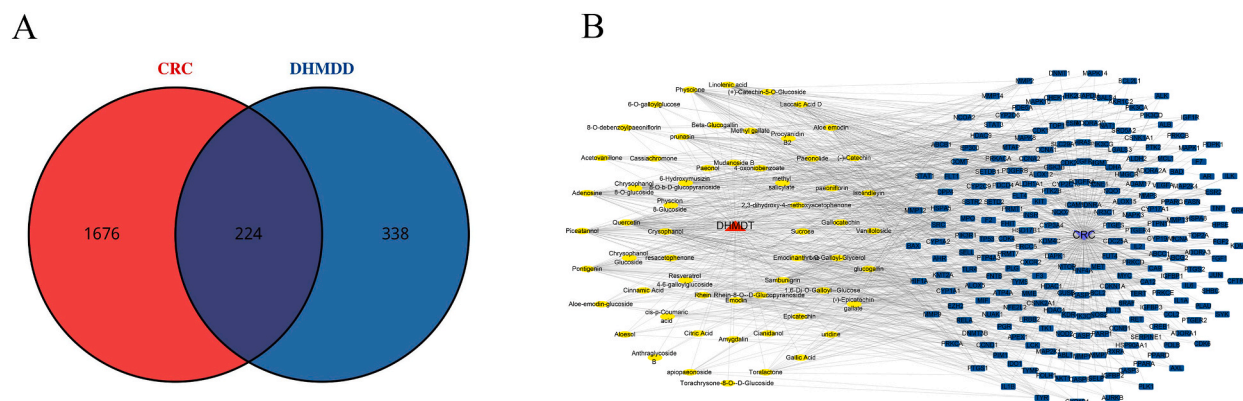
A

B

Fig. 2. (A)the Venn Diagram between constituents-related targets and CRC-related targets; (B)The network of 53 constituents and overlapped CRC-related targets.

A

B

C

Fig. 3. (A) Protein-Protein Interaction Networks of potential targets; (B) topological analysis of potential targets; The color from light to dark indicates the Degree value from small to large; (C) the Interaction Networks of ten core targets.

3.3. Experimental validation in vitro

3.3.1. DHMDD effect on colorectal cancer cells proliferation

The concentration-dependent toxic effect of DHMDD on HCT116 cells was observed to gradually intensify by CCK-8 assay, as indicated by the increasing inhibition rate. Based on the curve in Fig. 6A, the IC50 of DHMDD on HCT116 cells was estimated to be approximately 17.8 mg/ml. These findings confirm the toxic effect of DHMDD on HCT116 cells, and the concentration corresponding to half inhibition rate was selected for subsequent experiments. The same effect was also detected in a colony formation assay (Fig. 6B). The DHMDD group exhibited a significant decrease in the number of cell clones compared to the control group, and this difference was statistically significant ($p < 0.01$).

3.3.2. DHMDD effect on colorectal cancer cells invasion and migration

In view of cellular invasion and migration were the important characteristics of tumor metastasis, HCT116 cells were observed for assessing the capability of invasion and migration by using Matrigel-coated transwell plates and wound healing assay. The results demonstrated a significant reduction in the invasion and migration abilities of Colorectal Cancer Cells following treatment with DHMDD (Fig. 6C and D).

3.3.3. DHMDD induced apoptosis via PI3K/AKT signaling pathway

The mRNA expression levels of key targets, including AKT1, PI3K, PIK3R1, and PIK3CA, were assessed using qRT-PCR to investigate the effect of DHMDD. The DHMDD group exhibited significantly higher mRNA expression levels of AKT1, PI3K, PIK3R1, and
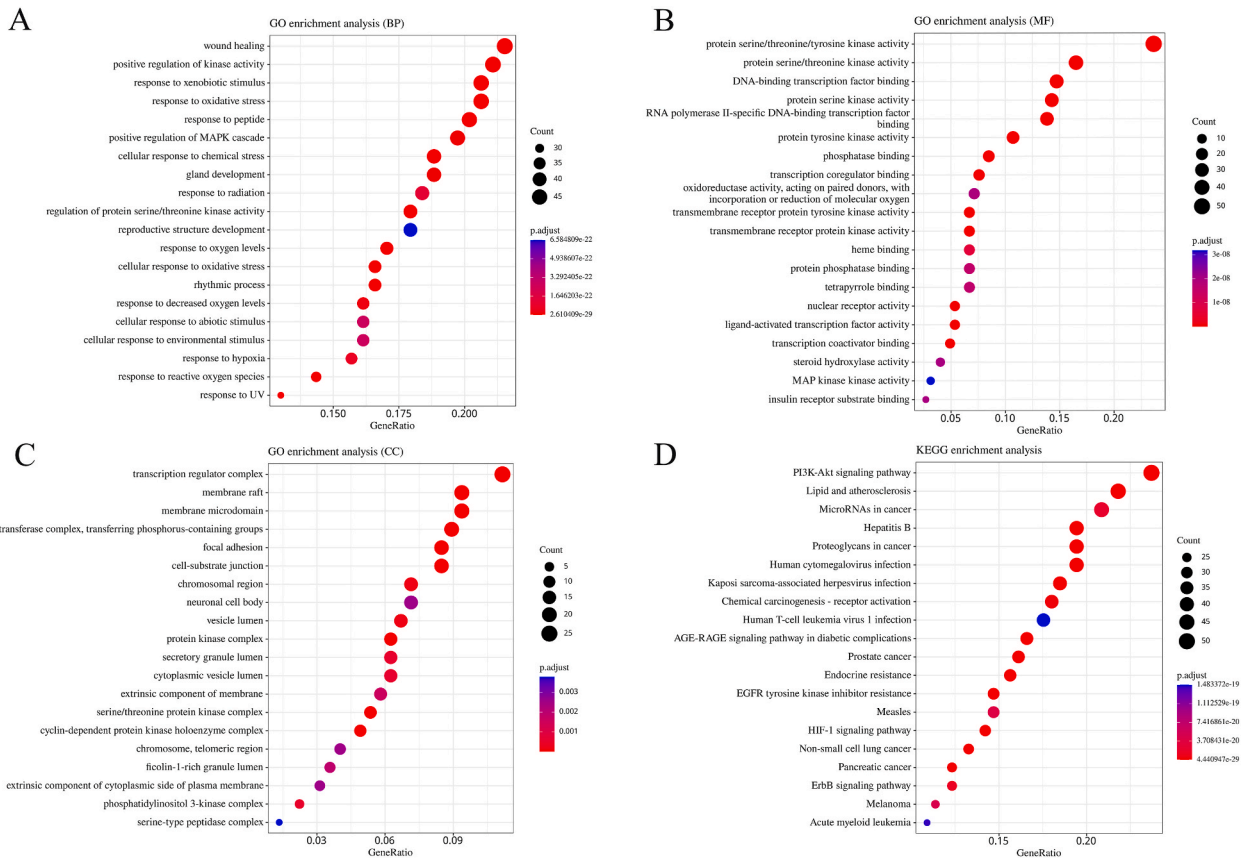
Fig. 4. Enrichment analysis of potential targets from main active ingredients of DDMDD: (A) Gene ontology terms for biological process; (B) Gene ontology terms for molecular function; (C) Gene ontology terms for cellular component; (D) KEGG pathways.
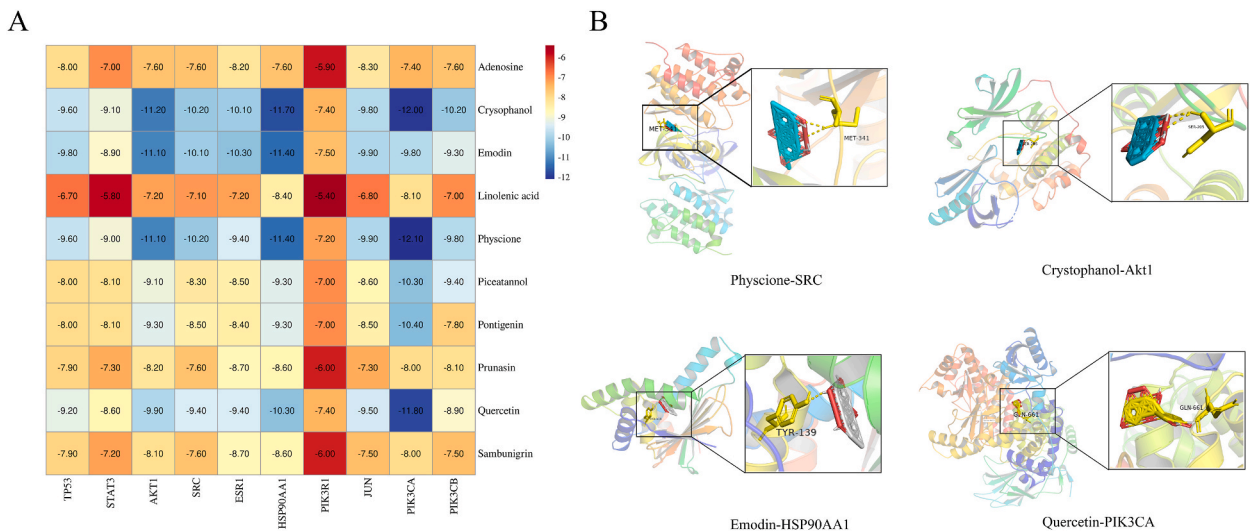

Fig. 5. (A) Molecular docking heat map; (B)Schematic diagram of molecular docking.

PIK3CA compared to the control group (p < 0.05), as shown in Fig. 6E. The results of western blotting analysis showed the AKT1 protein expression in the DHMDD group was not significantly different from that in the control group, while the expression of p-AKT1 was significantly decreased (p < 0.05). The DHMDD group exhibited higher Bax protein expression compared to the control group (p < 0.05), and there was no statistically significant difference in Bcl-2 protein expression between the two groups. However, the ratio of
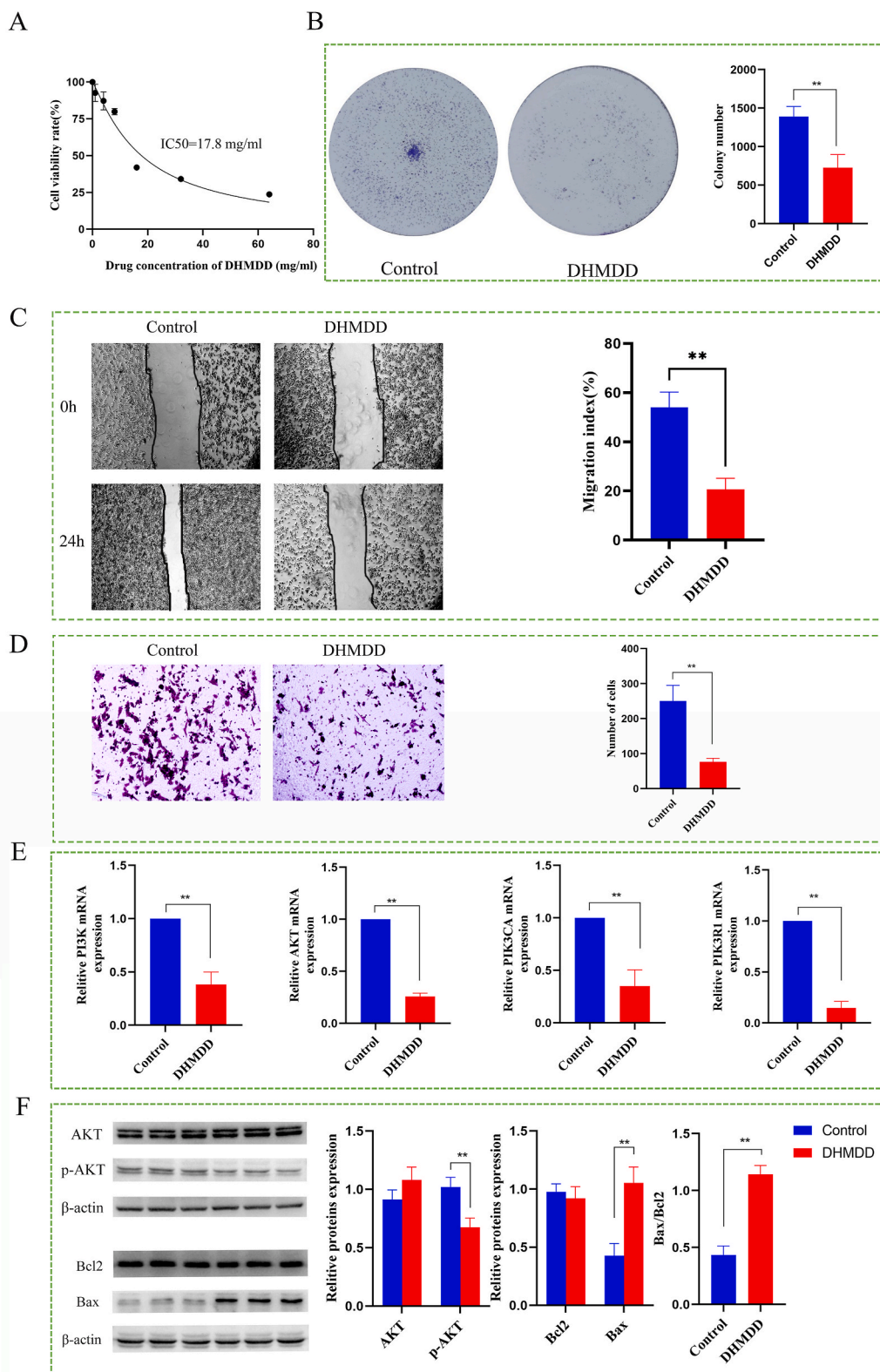
Fig. 6. (A) The graph of DHMDD inhibiting HCT116 growth. (B) Effect of DHMDD on CRC cell clonal formation (**$p < 0.01$). (C) Effect of DHMDD on CRC cell invasion (**$p < 0.01$).(D) Effect of DHMDD on CRC cell migration (**$p < 0.01$).(E) Effect of DHMDD on PI3K, AKT1, PIK3CA and PIK3R1 mRNA expression.(F) Effect of DHMDD on AKT1, p-AKT1, Bcl 2 and Bax protein expression (*$p < 0.05$, **$p < 0.01$).

Bax/Bcl-2 proteins was higher in the DHMDD group than in the control group (p < 0.05) (Fig. 6F).

4. Discussion

Colorectal cancer is a highly prevalent and lethal malignancy worldwide. The primary treatment modalities for this disease include surgery, radiotherapy, chemotherapy, and targeted therapy. However, these approaches have limitations such as extensive surgical trauma, significant side effects of radiotherapy and chemotherapy, and limited efficacy of targeted therapy based on the molecular profile of the tumor. Therefore, the current research focus is to discover novel treatment strategies that can enhance the quality of life and survival rates in patients with colorectal cancer [4]. DHMDD is a traditional Chinese medicine formulation comprising Rheum potaninii Losinskaja, Moutan Cortex, Prunus persica L. Batsch, Semen Benincasae, and Natrii Sulfas. It is known for its efficacy in treating intestinal diseases. However, the precise mechanism of action of DHMDD in the treatment of colorectal cancer remains incompletely elucidated.

In this study, UPLC-Q/TOF-MS technology was employed to analyze and identify 81 compounds present in DHMDD. To establish the correlation between these compounds and the clinical efficacy, network pharmacology methods were utilized to screen and identify 224 targets. The network pharmacology study found the connection between the active ingredients of traditional Chinese medicine, potential targets and diseases, predicted the key targets of traditional Chinese medicine in the treatment of related diseases, and combined with molecular docking, could preliminarily verify the stability of the combination of effective active ingredients and key targets. Among the 52 active ingredients found in DHMDD, this study revealed that quercetin, physcione, pontigenin, crysophanol, linolenic acid, piceatannol, adenosine, emodin, sambunigrin, and prunasin are the main active ingredients derived from DDMDD for the treatment of colorectal cancer, as determined from a network pharmacology perspective.

The PPI network screening results show that AKT1, ESR1, HSP90AA1, JUN, PIK3CA, PIK3CB, PIK3R1, SRC, STAT3 and TP53 are the top ten targets. AKT1 is a serine/threonine protein kinase that plays an important role in regulating cell growth, proliferation, and survival [28]. ESR1 is the most important regulatory gene in the occurrence, development and metastasis of CRC, and is a potential mechanism for inducing CRC [29]. HSP90AA1 is closely linked to tumor cell cycle, apoptosis, invasion, and differentiation, making it a key protein in CRC treatment [30]. JUN, a member of the AP-1 family of transcription factors, plays a crucial role in cell proliferation, apoptosis, and transcriptional regulation. Abnormal expression of JUN in colorectal cancer is linked to tumor invasion and metastasis [31]. The PI3K family, a class of phosphatidylinositol kinases, plays a vital role in cell proliferation, survival, and metabolic regulation. PIK3CA, PIK3CB, and PIK3R1, important members of the PI3K family, are frequently mutated and abnormally activated in colorectal cancer [32]. SRC, a non-receptor tyrosine kinase, is involved in cell signaling and transcriptional regulation. Abnormal activation of SRC is associated with tumor growth, invasion, and metastasis [33,34]. STAT3, a transcription factor, plays a critical role in cell proliferation, survival, and immune regulation [35–38]. TP53, a tumor suppressor gene, is essential for apoptosis, DNA repair, and cell cycle regulation. Mutations and loss of function in TP53 are common in various cancers [39,40]. These targets have significant implications in the onset and progression of colorectal cancer. The therapeutic efficacy of DHMDD may be closely related to its regulatory effects on these targets.

KEGG pathway enrichment analysis showed that DHMDD treatment of CRC may involve PI3K/Akt signaling pathway, AGE-RAGE signaling pathway, HIF-1 signaling pathway and other pathways in cancer. Among them, the highly enriched PI3K/Akt signaling pathway is one of the most important signaling pathways in various cancers, and its abnormal activation can promote the development of tumors. The PI3K/Akt signaling pathway is frequently mutated and abnormally activated in various tumor cells [41–43]. The activation of PI3K/AKT signaling pathway is involved in the regulation of biological processes related to cell proliferation, differentiation, motility, survival and various metabolic processes [44,45]. Molecular docking showed that DHMDD had the lowest binding energy and the most stable conformation with PI3KCA and AKT1. Therefore, based on GO function, KEGG pathway enrichment analysis and molecular docking results, the core target of the PI3K/Aktsignalingpathway was selected for cell verification in this study.

We conducted in vitro experimental studies to investigate the potential mechanism of DHMDD in colorectal cancer, based on the results of network pharmacology. Cell experiments demonstrate that DHMDD can enhance apoptosis in colorectal cancer cells by modulating the PI3K/Akt pathway. This suggests that DHMDD has the potential to suppress the growth and metastasis of colorectal cancer by modulating the PI3K/Akt signaling pathway. These findings offer valuable insights into the potential mechanism of DHMDD as a therapeutic approach for colorectal cancer. This also validates the reliability of network pharmacological predictions and molecular docking results.

In summary, this study demonstrates that DHMDD has the potential to treat colorectal cancer through a multi-component and multi-target approach, as evidenced by network pharmacology analysis and cell experiments. The findings of this study provide additional support for considering DHMDD as a potential therapeutic drug for colorectal cancer. Nevertheless, this study has certain limitations. First of all, this study primarily relies on in vitro experiments and lacks validation in clinical trials and animal models. Therefore, additional studies are required to verify the effectiveness and safety of DHMDD in real clinical settings. Secondly, this study solely examined the pharmacological activity and a portion of the therapeutic mechanism of DHMDD, necessitating further comprehensive investigation into its overall mechanism of action in colorectal cancer.

Overall, our in-depth exploration of the pharmacological activity and therapeutic mechanism of DHMDD revealed its potential to inhibit the growth and metastasis of colorectal cancer through the regulation of the PI3K/Akt signaling pathway. These findings offer novel insights for the clinical application of DHMDD and are expected to stimulate further research and development in the field of colorectal cancer treatment. Future research can explore the synergistic effects of combining DHMDD with other treatments to enhance therapeutic outcomes. Additionally, research on drug metabolism, drug interactions, as well as the toxicological and side effects of DHMDD, is imperative. By employing a comprehensive approach that integrates multidisciplinary research methods, we can achieve a

more comprehensive understanding of the mechanism underlying DHMDD's efficacy in colorectal cancer treatment, thereby offering more effective therapeutic strategies for clinical practice.

5. Conclusion

This study comprehensively evaluated the pharmacological activity and therapeutic mechanism of DHMDD in treating colorectal cancer using network pharmacology and in vitro experiments. The research findings demonstrate that DHMDD inhibits the proliferation, invasion and migration of colorectal cancer cells and promotes cell apoptosis by regulating the PI3K/Akt signaling pathway. This finding holds significant importance in enhancing our understanding of the anti-colorectal cancer mechanism of DHMDD. DHMDD, a traditional Chinese medicine, shows potential as an adjuvant treatment for colorectal cancer, offering significant efficacy and minimal side effects. However, despite the important findings obtained in this study, there are still certain limitations. Future research should focus on further verifying the therapeutic efficacy and safety of DHMDD, utilizing clinical experiments and animal models. Furthermore, additional studies are needed to fully comprehend the therapeutic effects of DHMDD in colorectal cancer by investigating other mechanisms. In conclusion, this study establishes a scientific foundation for considering DHMDD as a potential drug for colorectal cancer treatment. The findings of this study offer significant support for considering DHMDD as a potential drug for colorectal cancer treatment and provide valuable guidance for further research and development of DHMDD's clinical applications. This will help enhance the treatment outcomes and quality of life for individuals diagnosed with colorectal cancer, while also presenting novel concepts and strategies for personalized treatment within the field of colorectal cancer.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Xinghua Li: Writing – review & editing, Writing – original draft, Conceptualization. **Xinyue Liu:** Writing – review & editing, Validation. **Fan Yang:** Validation. **Tianwei Meng:** Data curation. **Xiang Li:** Validation. **Yan Yan:** Validation. **Keyuan Xiao:** Validation, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32136.

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