

Exploring the Potential Mechanism of "Yujin Powder" in Treating Canine Cholecystitis Based on Network Pharmacology and Molecular

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ABSTRACT

Objective: To predict the core active ingredients, potential targets, and signaling pathways of "Yujin Powder" in treating canine cholecystitis using network pharmacology and molecular docking, and to explore its underlying mechanism. **Methods:** Active ingredients of "Yujin Powder" (including Curcuma, Coptis chinensis, Rheum palmatum, etc.) and their targets were retrieved from the TCMSP database. Disease targets related to canine cholecystitis were obtained from Genecards and OMIM databases. R software was used to identify drug-disease intersection targets and construct a protein-protein interaction (PPI) network. GO functional and KEGG pathway enrichment analyses were performed using the R package clusterProfiler. Molecular docking verification was carried out using AutoDock Vina. **Results:** A total of 60 active ingredients and 187 corresponding targets were identified for "Yujin Powder". There were 1070 disease-related targets for canine cholecystitis, resulting in 62 intersection targets. The PPI network identified core targets such as TNF, IL6, IL1B, EGFR, MMP9, and TP53. GO enrichment analysis revealed that the biological processes were primarily involved in cellular response to lipopolysaccharide and regulation of smooth muscle cell proliferation. KEGG pathway enrichment suggested close associations with cholecystitis-related pathways such as the IL-17 signaling pathway and AGE-RAGE signaling pathway. Molecular docking indicated that the core ingredient MOL000098 (quercetin) bound to the core target TP53 with a binding energy of -6.632 kcal/mol. **Conclusion:** Yujin Powder may exert therapeutic effects on canine cholecystitis through multiple active ingredients like quercetin, acting on core targets such as TP53, TNF, and IL6, and regulating multiple pathways including the IL-17 signaling pathway. This suggests roles in anti-inflammation, immune regulation, and protection of biliary smooth muscle. This study preliminarily reveals the multi-component, multi-target, and multi-pathway characteristics of Yujin Powder in treating canine cholecystitis, providing a theoretical basis for subsequent experimental validation and clinical application.

KEYWORDS

Yujin Powder; Canine cholecystitis; Network pharmacology; Molecular docking

1 Introduction

Canine cholecystitis is a common digestive system disease in dogs, characterized by gallbladder inflammation, pain, and dysfunction. Its pathogenesis is associated with factors such as bacterial infection and bile stasis ^[1]. Current treatments primarily rely on antibiotics and surgery, but long-term medication use can easily lead to drug resistance ^[2]. Traditional Chinese Medicine (TCM) holds unique advantages in treating gallbladder diseases. "Yujin Powder" (Yujin San), a traditional formula composed of Curcuma (Yujin), Coptis chinensis (Huanglian), Rheum palmatum (Dahuang), etc., functions to clear heat, resolve dampness, soothe the liver, and promote bile flow. It has shown good efficacy in clinical applications for canine cholecystitis, but its mechanism of action remains unclear ^[3]. Network pharmacology can systematically analyze the multi-component, multi-target, and multi-pathway interactions of TCM formulas. Combined with molecular docking technology, which can verify binding activity, it serves as a powerful tool for modern TCM research ^[4]. This study aims to explore the potential mechanism of "Yujin Powder" in treating canine cholecystitis using network pharmacology and molecular docking, providing direction for experimental research.

2 Materials and Methods

2.1 Acquisition of Active Ingredients and Drug Targets

Active ingredients of the constituent herbs of "Yujin Powder" (Curcuma, Coptis chinensis, Rheum palmatum, Gardenia, etc.) were retrieved from the TCMSP database (<https://tcmsp-e.com>). Core active ingredients were screened based on Oral Bioavailability (OB) $\geq 30\%$ and Drug-likeness (DL) ≥ 0.18 . Corresponding targets for these core active ingredients were then obtained.

2.2 Acquisition of Disease Targets

Disease-related targets for cholecystitis were retrieved from the Genecards database (<https://www.genecards.org>) using the keyword "cholecystitis".

2.3 Screening of Intersection Targets

R software (version 4.0.3) was used to identify the intersection between drug targets and disease targets. The R

package ggvenn (version 0.1.19) was used to draw a Venn diagram.

2.4 Construction of PPI Network

The intersection targets were imported into the STRING database (<https://string-db.org>), with the species set to *Homo sapiens*, to construct a PPI network. Cytoscape software (version 3.10.4) was used for topological analysis to screen core targets based on degree ranking.

2.5 Enrichment Analysis

The R package clusterProfiler (version 4.14.6) was used to perform Gene Ontology (GO) functional enrichment analysis (including Biological Process (BP), Cellular Component (CC), Molecular Function (MF)) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis on the intersection targets. A p.adjust value < 0.05 was set as the significance threshold.

2.6 Molecular Docking

The 3D structures of core active ingredients were downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>). Protein structure information for core targets was retrieved from the UniProt database (<https://www.uniprot.org/>), and protein structures were downloaded from the PDB database (<https://www.rcsb.org>). Molecular docking was performed using AutoDock Vina (version 1.2.7). A binding energy < -5 kcal/mol indicated good binding activity.

3 Results

3.1 Active Ingredients and Targets of the Drug

The main components of Yujin Powder are Curcuma, Rheum palmatum, Coptis chinensis, and Gardenia. We searched the TCMSP database for these four medicinal materials and identified 222 compounds in Curcuma, 92 in Rheum palmatum, 48 in Coptis chinensis, and 98 in Gardenia. Using the screening criteria of OB ≥ 30% and DL ≥ 0.18, we obtained 15 active ingredients for Curcuma, 16 for Rheum palmatum, 14 for Coptis chinensis, and 15 for Gardenia. The distribution of the top 20 core active ingredients is shown in Figure 1. A total of 187 protein targets corresponding to the active ingredients were also obtained.

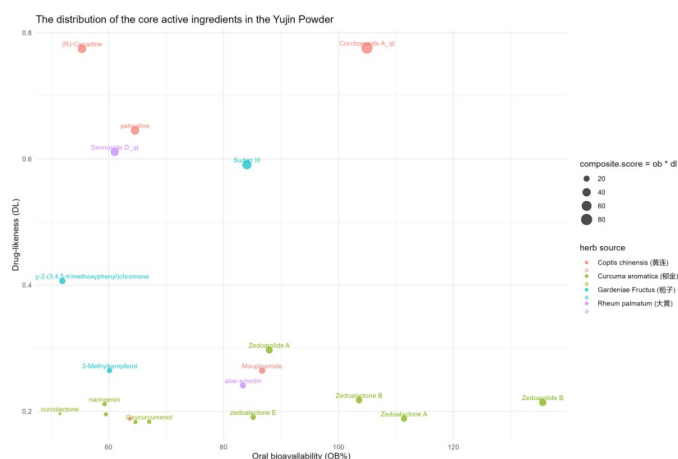


Figure 1 Distribution of core active ingredients in Yujin Powder

A dot plot showing the top 20 core active ingredients. The X-axis represents oral bioavailability (OB), the Y-axis represents Drug-likeness (DL), and the activity score = OB * DL.

3.2 Disease Targets and Intersection Targets

A total of 1070 targets related to canine cholecystitis were obtained from the Genecards database. The intersection of drug targets and disease targets yielded 62 common targets, as shown in the Venn diagram in Figure 2.

The Venn diagram shows the intersection between the active ingredient targets of "Yujin Powder" and the disease targets of canine cholecystitis. The left circle represents drug targets (187), the right circle represents disease targets (1070), and the overlapping section represents the intersection targets (62).

3.3 Construction of the Active Ingredient-Disease Target Network

We constructed an "active ingredient-disease target" network based on the intersection of active ingredients from the four components of Yujin Powder and the disease targets. The results are shown in Figure 3. We found that MOL000098 (quercetin) had the most connections to cholecystitis-related targets.

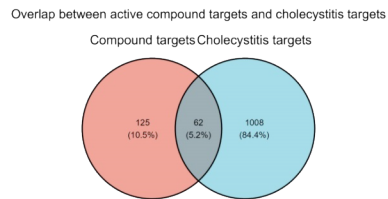


Figure 2 Venn diagram of the intersection targets between Yujin Powder and canine cholecystitis

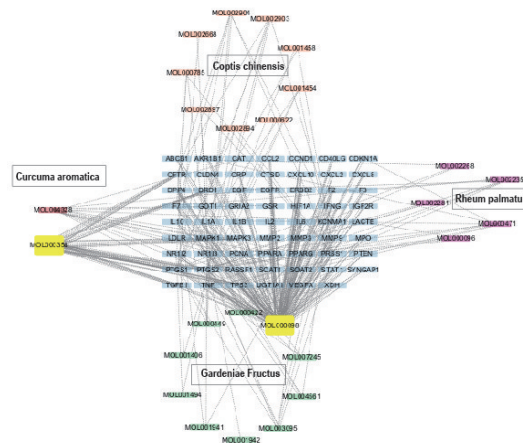


Figure 3 Network diagram of active ingredients and disease targets

The network diagram shows the relationships between the active ingredients of the four components and the intersection targets of cholecystitis. The center represents cholecystitis-related targets, the left represents Curcuma active ingredients, the right represents Rheum palmatum active ingredients, the top represents Coptis chinensis active ingredients, and the bottom represents Gardenia active ingredients.

3.4 PPI Network Analysis

The 62 intersection targets were imported into the STRING database to construct a PPI network. The network contained 61 nodes and 646 edges, with an average number of neighbors of 21.8. Using Cytoscape software, the top 6 targets by degree value were selected as core targets, as shown in Figure 4, including TNF, IL6, IL1B, EGFR, MMP9, and TP53.

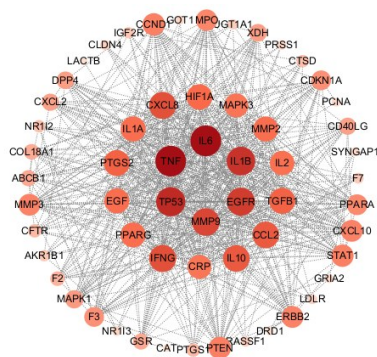


Figure 4 PPI network of intersection targets

The PPI network diagram shows the protein-protein interactions among the intersection targets. Nodes represent target proteins, and edges represent interaction relationships. The node size and color are graded according to the Degree value.

3.5 GO Enrichment Analysis

GO enrichment analysis yielded 1654 terms, including 1541 Biological Process (BP) terms, 82 Molecular Function (MF) terms, and 32 Cellular Component (CC) terms. The top-ranked terms included pathways related to cholecystitis, such as 'cellular response to lipopolysaccharide', 'regulation of smooth muscle cell proliferation', 'cytokine receptor binding', and 'cytokine activity', as shown in Figure 5.

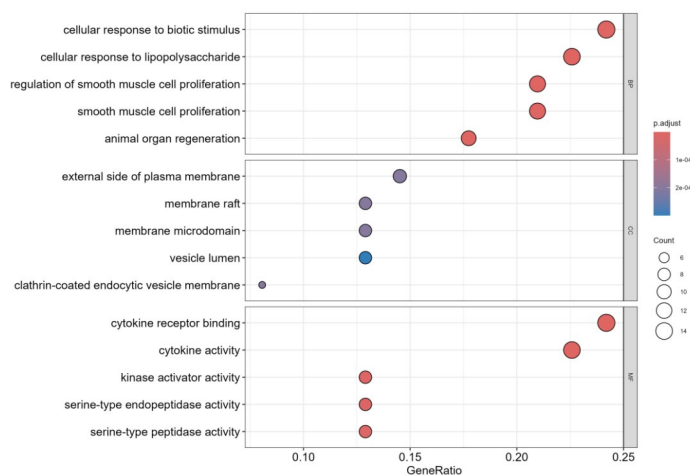


Figure 5 GO enrichment analysis of intersection targets

The GO enrichment plot shows the main functional categories of the intersection targets. The X-axis represents the GeneRatio, and the Y-axis represents the GO terms. The bubble size indicates the number of targets, and the color indicates the P-value.

3.6 KEGG Pathway Enrichment Analysis

KEGG enrichment analysis obtained 160 pathways. The top 10 pathways with the lowest FDR are displayed, revealing cholecystitis-related pathways such as the "IL-17 signaling pathway" and "AGE-RAGE signaling pathway", as shown in Figure 6.

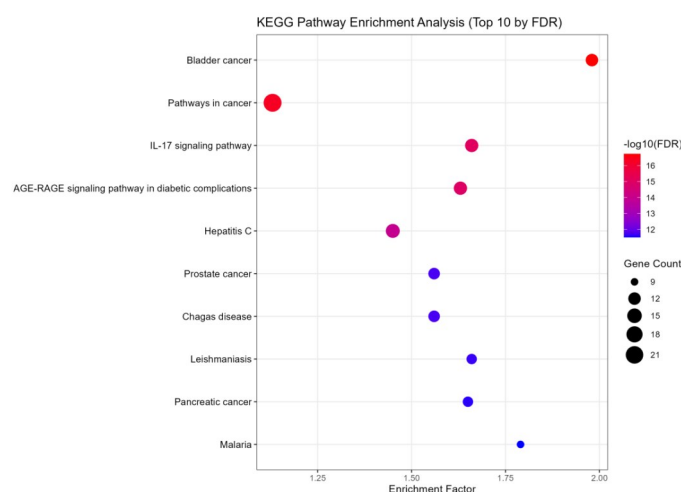


Figure 6 KEGG pathway enrichment bubble chart of intersection targets

The KEGG pathway enrichment bubble chart shows the main signaling pathways involved by the intersection targets. The bubble size indicates the number of targets, and the color indicates the P-value.

3.7 Molecular Docking

The core ingredient MOL000098 (quercetin) was selected for verification via molecular docking. We found that the targets of quercetin included IL1B, IL6, EGFR, MMP9, TNF, and TP53. A search in the UniProt database revealed that only TP53 had structural information available. TP53 (PDB: 1TUP) was selected for molecular docking with quercetin. The docking binding energy was -6.632 kcal/mol, and the docking structure is shown in Figure 7.

The molecular docking simulation diagram of quercetin with protein 1TUP (TP53) was generated using PyMOL based on the AutoDock Vina results. Green: 1TUP (TP53); Red: quercetin.

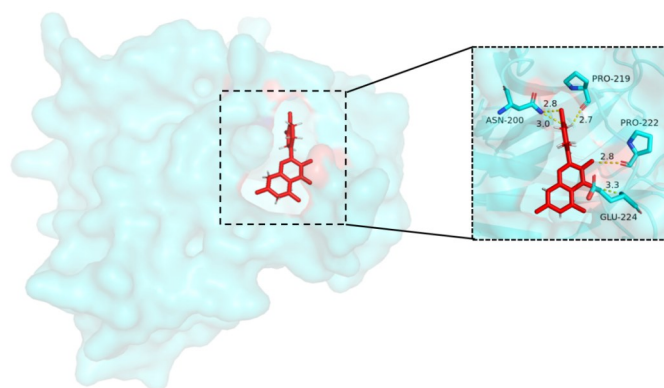


Figure 7 Molecular docking simulation diagram of quercetin with 1TUP (TP53)

4 Discussion

This study integrated network pharmacology and molecular docking technology to preliminarily reveal the potential material basis and mechanism of action of Yujin Powder in treating canine cholecystitis. The findings indicate that Yujin Powder exerts synergistic effects through multiple active ingredients, multiple targets, and multiple signaling pathways, reflecting the holistic regulation and multi-pathway intervention characteristics of TCM formulas. By predicting the mechanism of "Yujin Powder" in treating canine cholecystitis using network pharmacology, it was found that its core components, such as quercetin, likely act on targets like TNF, IL6, IL1B, EGFR, MMP9, and TP53. GO enrichment highlighted "cellular response to lipopolysaccharide" as a significant biological process. In cholecystitis, bacterial infection often involves lipopolysaccharide (LPS), which can trigger inflammatory responses. The involvement of cytokine activity and regulation of smooth muscle cell proliferation are also highly relevant to the pathology of cholecystitis, involving inflammation and potential smooth muscle dysfunction in the biliary system. The KEGG pathways identified, such as IL-17 signaling and AGE-RAGE signaling, are known to be involved in inflammatory processes. Molecular docking results further verified the binding activity between the core ingredient (quercetin) and the core target (TP53), providing a focus for experimental research. A limitation of this study is its reliance on database predictions; thus, further validation through *in vitro* and *in vivo* experiments is necessary.

References

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