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Study on the Pharmacological Mechanism of Yinqiao Antipyreti Granules Against Influenza Based on Network Pharmacology

Suishan Qiu, Lianfang Xue, Huiting Zhong, Leping Li, Hui Liu*

Department of Pharmacy, The First Affiliated Hospital of Jinan University, Guangzhou, China

Email address:

494490817@qq.com (Suishan Qiu), xlf18666@163.com (Lianfang Xue), hinsabc@163.com (Huiting Zhong), 1473203893@qq.com (Leping Li), liu_ui@hotmail.com (Hui Liu)

*Corresponding author

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Abstract: Objective: To explore the anti-influenza pharmacological mechanism of Yinqiao Antipyreti Granules by using network pharmacology. Method: Retrieve the main active ingredients, corresponding targets and target genes of Yinqiao Antipyreti Granules on the website of Chinese Medicine System Pharmacology Technology Platform (TCMSP), obtain the relevant target genes of influenza through the Human Gene Database (Gene Cards), and transfer the drugs The target of the active ingredient is mapped to the influenza target, and the intersection target obtained is the predicted target of Yinqiao Ture granules against influenza. Cytoscape 3.8.0 software was used to construct a drug active ingredient-intersection target network model, and key active ingredients were selected. Use the STRING website to construct an intersection target protein interaction network (PPI) and select key target genes. Enriched analysis of GO and KEGG pathways using Metascape on intersection targets. Results: There are 136 active ingredients in Yinqiao Antipyretic Granules and 131 anti-influenza-related targets. Conclusion: The main components of Yinqiao Antipyretic Granules are quercetin, luteolin, naringenin, formononetin, IL-6, STAT3, MAPK14, CASP3 and other gene targets, to suppress inflammatory response, regulate immunity, reduce the symptoms of influenza, and thus play an anti-influenza therapeutic role.

Keywords: Yinqiao Antipyreti Granules, Influenza, Network Pharmacology

1. Introduction

Influenza is an acute respiratory infectious disease caused by influenza virus. The seasonal influenza that breaks out globally every year can cause 5% to 15% of the world's population to become sick, and the number of deaths is about 300,000 to 500,000, A serious threat to human health. Influenza has the characteristics of high incidence, rapid onset, rapid spread and strong variability [1].

Yinqiao Antipyretic Granule (YQAG) is a modified classic TCM formula that is derived from the Yinqiao Powder and Liuyi Powder in the Item Differentiation of Warm Febrile Disease and Xuanming Lunfang respectively. Yinqiao Antipyretic Granule (YQAG, composed of Radix Isatidis (RI), Mint (MT), Licorice (LC), Cyrtomium Rhizome (CR), Honeysuckle (HY), Bitter Apricot Kernels (BAK), Phragmites Communis (PC), Fructus Arctii (FA), Forsythia Suspensa (FS)). YQAG has been prescribed to treat various causes of fever, suchasexogenous wind heat, Chickenpox, measles, mumps, damp heat and internal depression, cough and sore throat, sore boils and swelling pain, frequent urination and urgency in China.

Previous studies [2, 3] have illustrated that YQAG possesses heat-clearing, antiendotoxin and draining dampness activities. Among these herbal medicines, RI and HY are a commonly used traditional Chinese medicine (TCM). Many studies have proven that emodin has good clinical value in the treatment of influenza and cold. In recent years, much attention has been paid to TCM monomers and compounds. Studies have found that chlorogenic acid in

honeysuckle has antiviral effects. Antivirus potency of Radix Isatidis extract has been provided by many literatureas. TCM formulas are complex combinations with multiple components, multiple objectives and synergistic interactions among them.

Due to the complex chemical composition, it is difficult to study the role of the mixed system in the body. The complexity of TCM formulations makes it difficult to conduct a comprehensive study of TCM. With the development of network pharmacology, the research of single target has gradually changed to the research of overall and systematic regulation and has been used to study the functions of herbal formulations from a proteome or systematic level. The purpose of this study is to screen the bioactive components of YQAG, clarify its target, and further clarify the mechanism of network pharmacology in the treatment of influenza.

2. Methods

2.1. Collection of Bioactive Compounds and Potential Targets of YQAG

All components of YQAG were obtained from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php, Version: 2.3), a website that can provide information of herbal ingredients, structures. Parameters related to the absorption, distribution, metabolism and excretion (ADME) of herbal ingredients such as oral bioavailability (OB), drug similarity (DL) and half-life are also available in TCMSP. OB and DL were used to filter bioactive compounds of YQAG after data were collected from TCMSP. Only compounds with OB ≥ 30% and DL ≥ 0.18 were selected and were identified as target bioactive compounds for further study. In the Uniprot database and the national center for biotechnology information (NCBI) database (https://www.ncbi.nlm.nih.gov/), the species was restricted to "humans" and then the target bioactive compounds of YQAG were translated into the corresponding potential targets of YQAG.

2.2. Construction of Target Database of Influenze

The targets related to Influenza were acquired form GeneCards (https://www.genecards.org/). Gene Cards is a comprehensive, freely available database that provides information on targets related to diseases, mutations and polymorphisms, gene expression, gene function, pathways, protein-protein interactions, and so on. By searching the key word “influenze” the targets related to influenze were collected.

2.3. Prediction of Potential Anti-influenza Targets of YQAG

The gene symbols of candidate YQAG targets and Influenza-associated genes were uploaded into venny2.1 for the prediction of potential anti-influenza targets of YQAG.

2.4. Construction of Protein-Protein Interaction (PPI) Network and Selection of Key Targets

Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org/) was used to construct possible PPI network by uploading potential anti-influenza targets that related to YQAG. Species was limited to ”Homo sapiens” with a confidence score >0.7. The "Network Analyzer" in the software cytoscap3.8.0 was used to carry out topological analysis on PPI network, and the Degree of freedom was selected, with the target > 18 as the key targets.

2.5. Network Establishment and Pathway Analyses

Enrichment analysis and KEGG pathway construction of the key Target Gene Ontology (GO) Enrichment and Network was performed using R (version 3.6.0 for Windows). Pathways and networks were ranked according to the number of molecules in pathways and networks with a cut-off value ($p < 0.05$) for significantly enriched pathways/networks. Cytoscape 3.6.1 (http://www.cytoscape.org). Constructed a network of active components-key target-pathways of YQAG, and visually analyzes the relationship between components, targets and pathways.

3. Results

3.1. Composite Ingredients of YQAG

A total of 1321 compounds were collected from TCMSP. After ADME screening by OB≥ 30% and DL≥0.18, 136 compounds were identified as bioactive compounds of YQAG. Furthermore, 246 target genes of YQAG were colletioned from NCBI database. The results of bioactive compounds and target genes from herbs of YQAG are presented in Table 1.

Table 1. *Compounds and targets of YQAG.*

TCM	Number of compounds	Number of active ingredients	Number of Predict targets
Radix isatidis	169	39	91
Mint	164	10	110
Licorice	280	92	237
Cyrtomium Rhizome	34	7	80
Honeysuckle	236	23	207
Bitter Apricot Kernels	113	19	71
Phragmites Communis	31	1	27
Fructus Arctii	144	8	153
Forsythia Suspensa	150	23	209

3.2. Number of Influenza Targets

16,285 influenza-related target genes were retrieved from the Gene Cards database. A total of 706 targets with score≥1.87 were selected.

3.3. Intersection Target

A total of 131 common targets for drugs and diseases are obtained using the Venny online tools, as shown in Figure 1.

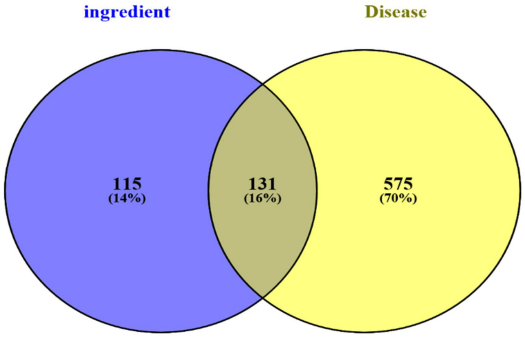

Figure 1. *Prediction of the potential target of YQAG against influenza venny diagram.*

3.4. Disease-Compound-Target-Network and Analysis

The component-target network was constructed by using Cytoscape software according to the active ingredients and intersection target in the active ingredients, "Degree" ranked in the top 10, including quercetin, luteolin, kaempferol, isoflavone, formononetin, licorice chalcone, naringin, granulin, isorhamnetin, medallion and beta carotene (Figure 2).

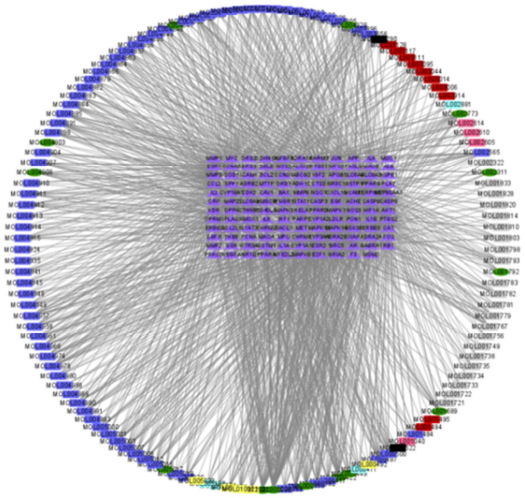

Figure 2. *The active component - target diagram of YQAG.*

3.5. Analysis of PPI Network

Import the Intersection target into the STRING database, export the data in TSV file format, and import the data into Cytoscape Software to construct the protein interaction network diagram (Figure 3), including 129 nodes, article1078 the edge. Then select the core target whose Degree value is greater than 18 to construct the core protein interaction network diagram (Figure 4).

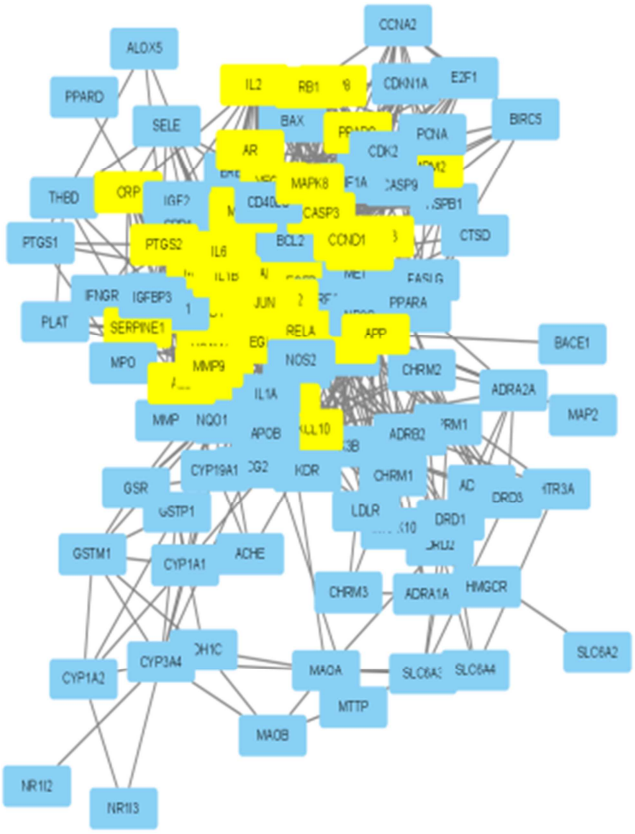

Figure 3. *PPI network diagram.*

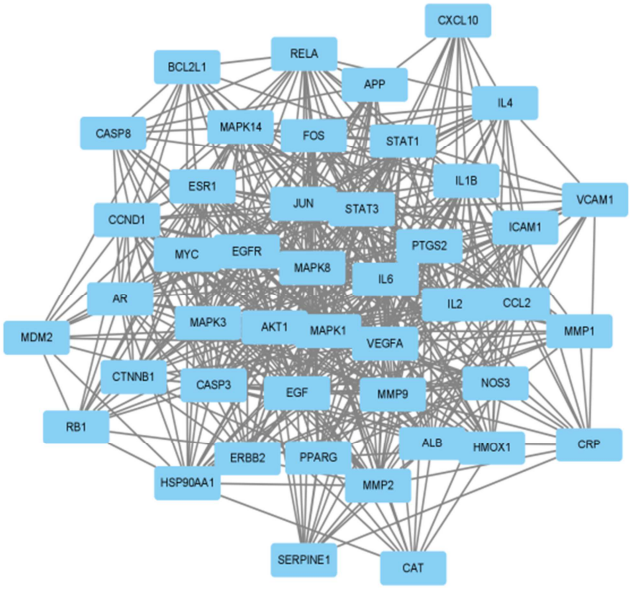

Figure 4. *Core PPI network diagram.*

3.6. Enrichment Analysis of GO and KEGG Pathway

The R software was used for GO enrichment analysis of 131 Intersection targets. According to the retention P≤0.05, the first 20 items were visually analyzed to obtain the bar chart.

The BP. of YQAG in the treatment of influenze include response to oxidative stress, response to nutrient levels, response to metal ion (Figure 5). CC include membrane raft, membrane microdomain, membrane region (Figure 6). MF include phosphatase binding, protein phosphatase binding, cytokine receptor binding, RNA polymerase II transcription factor binding (Figure 7).

Analysis of the KEGG pathway in the treatment of influenze by YQAG. include: Hepatitis B, infection, Hepatitis C, Measles, IL-17 signaling pathway, TNF signaling pathway, Kaposi sarcoma-associated herpesvirus infection, PI3K-Akt signaling pathway (Figure 8).

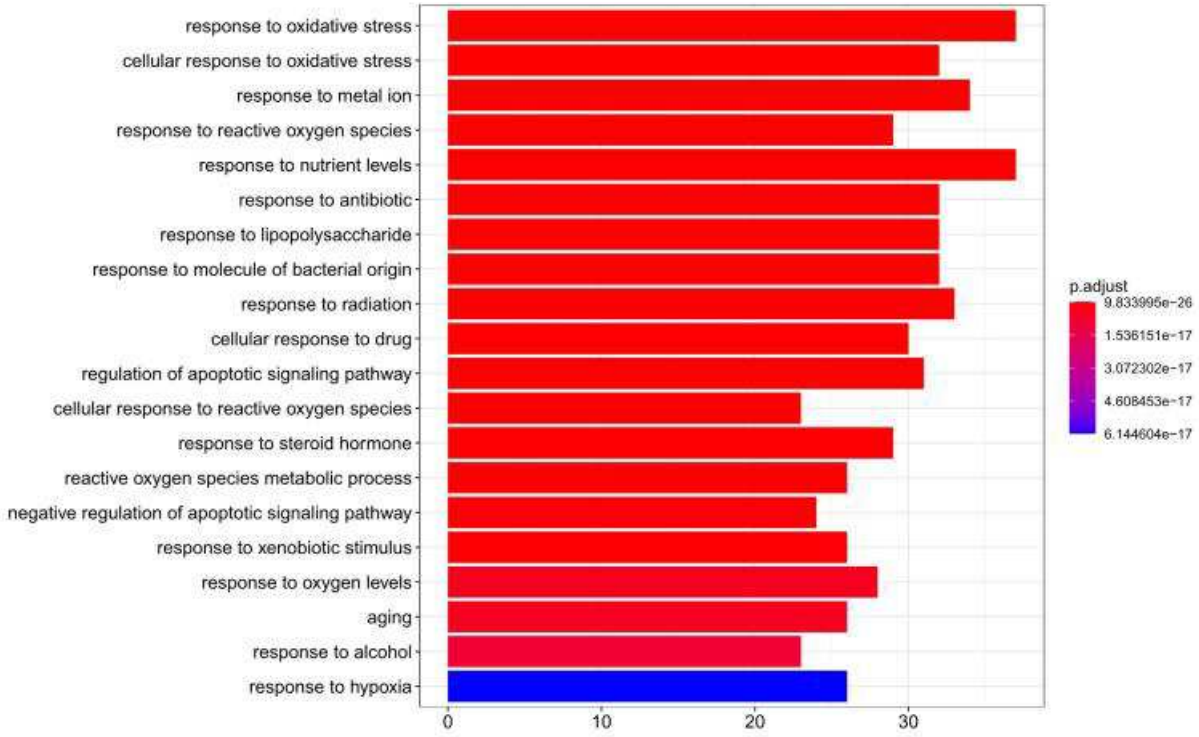

Figure 5. *The BP histogram of YQAG Anti-influenza.*

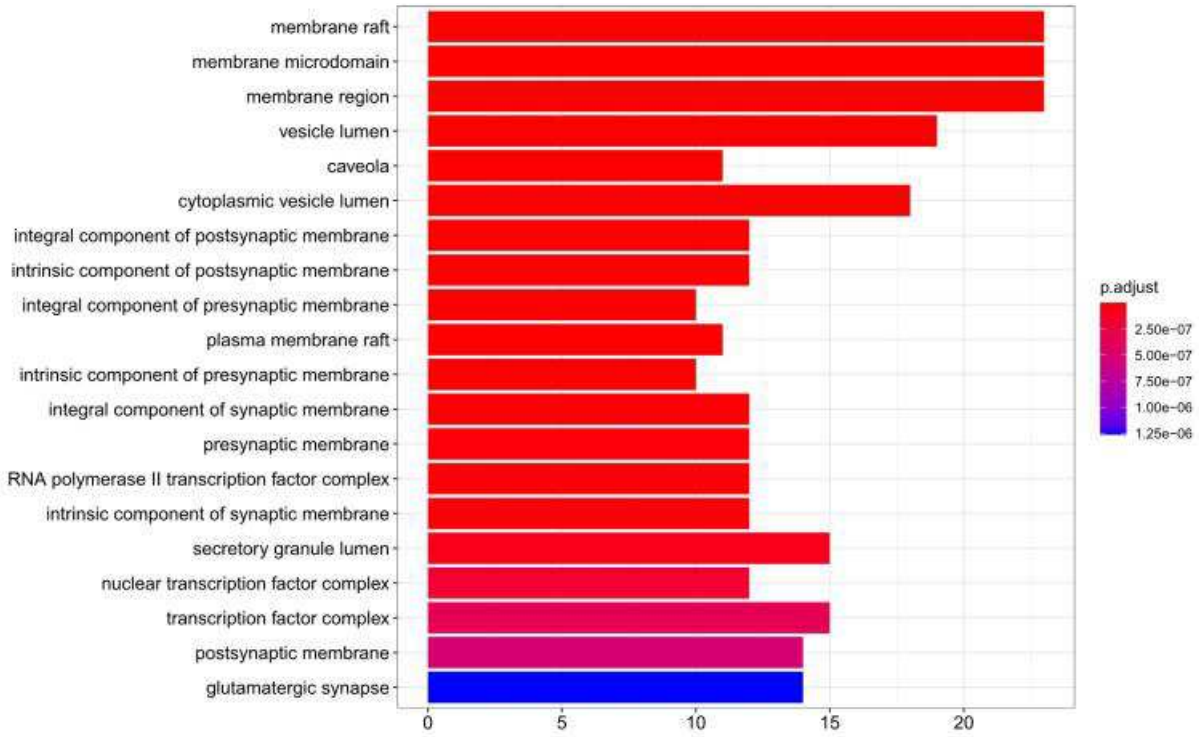

Figure 6. *The CC histogram of YQAG Anti-influenza.*

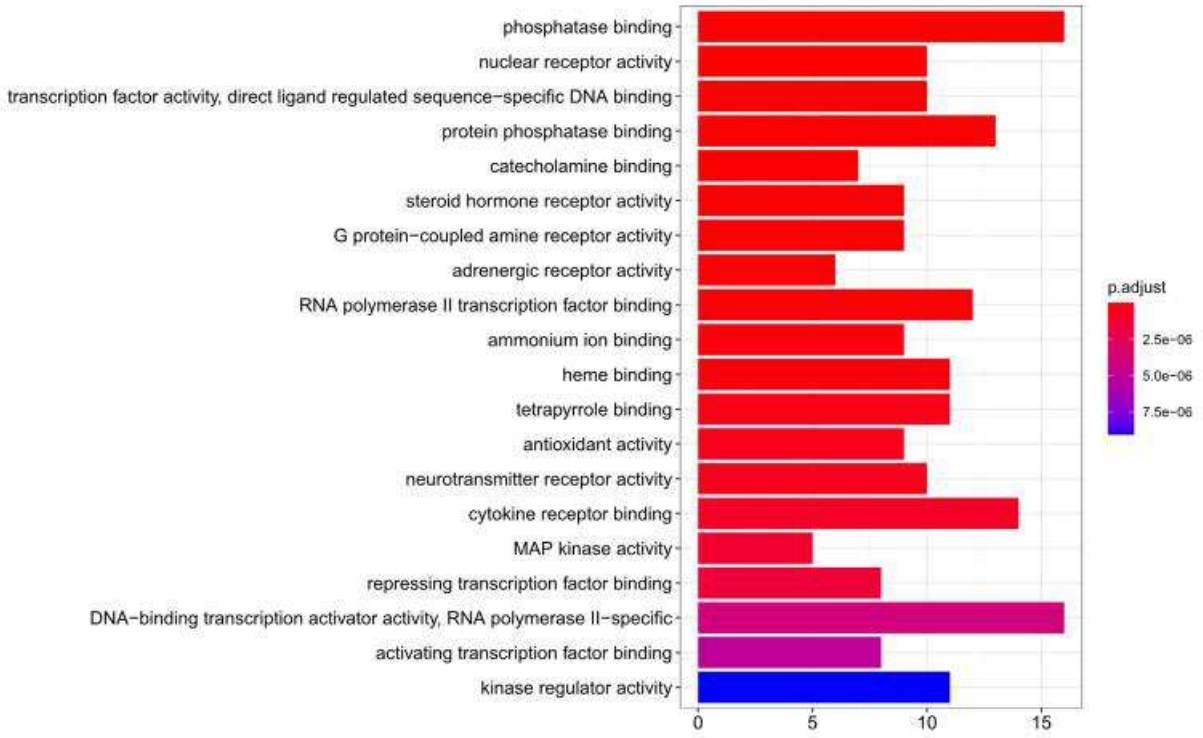

Figure 7. *The MF histogram of YQAG Anti-influenza.*

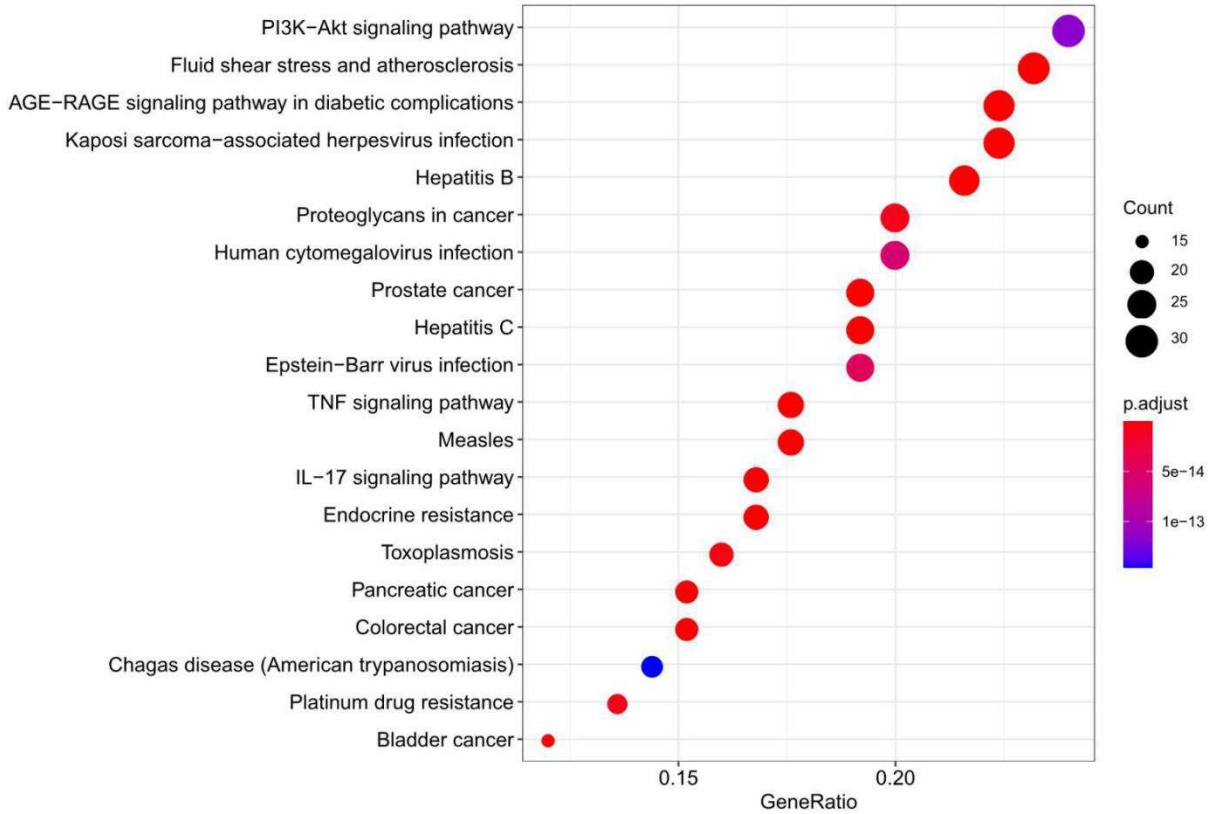

Figure 8. *KEGG diagram of YQAG Anti-influenza.*

3.7. Target-pathway Diagram

The obtained pathways and targets were imported into Cytoscape software, and a target-pathway diagram was drawn, as shown in Figure 9. Red represents the pathway, green represents the target, and the larger the shape, the more significant the enrichment. The core targets were IL-6, AKTI, CASP3, MAPK8, MMP9, MAPK10, CDK2, VEGFA,

MAPK14, STAT1, CCND1, STAT3 and ERBB2.

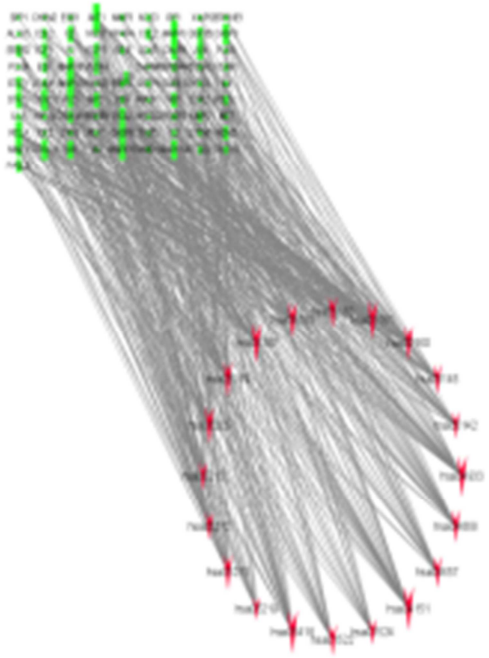

Figure 9. *Target-KEGG diagram of YQAG Anti-influenza.*

4. Discussion

Influenza is an acute respiratory infection caused by influenza virus. It can progress to severe influenza and even death due to acute respiratory distress syndrome or multiple organ failure. Influenza viruses are RNA viruses, belonging to the family paramyxoviridae, with three important proteins on the surface, such as hemagglutinin (HA), neuraminidase (NA) and matrix protein (M2). Influenza viruses enter cells through endocytosis, and viral genomes are transcribed and copied in the nucleus [4-6]. The replicated progeny virus particles spread through the respiratory mucosa and infect other cells. The released virus particles adhere to the host cells through sialic acid. NA hydrolyzes the sialic acid receptor, cutting off the connection between the virus particles and host cells, and promoting the release of progeny viruses to form new infections [7].

Studies have shown that quercetin has a protective effect on mice with LPS acute lung injury. Quercetin has anti-inflammatory effects by inhibiting NF-B signaling pathway and reducing the production of NF-B and ICAM-1 [8]. Luteolin has certain effects against virus, antioxidant and antibacterial. Luteolin inhibits viral RNA replication and prevents its synthesis to inhibit coxsackie virus A16 [9], and can down-regulate the expression of tumor necrosis factor α (TNF-α), IL-1, IL-6, and activate STAT to increase endogenous antiviral genes [10]. The abnormal activity of macrophages can produce a large number of inflammatory factors, such as TNF-α and interleukin etc., while luteolin can reduce the production of these inflammatory factors and prevent signal transcription from achieving anti-inflammatory effect [11]. Animal experiments showed that luteolin can reduce the release of tumor necrosis factor α (TNF-α) in the intercellular adhesion molecule-1 (ICAM-1) in the liver and inhibit the inflammation between lung tissues [12]. Kaempferol has antioxidant activity, scavenging oxygen free radicals and reducing hypoxia-induced tissue damage [13]. It also inhibits TNF-α, IL-6, IL-10, IL-1 and icAM-1 expression levels by reducing MAKP, NF-B pathways [14]. Naringenin has anti-inflammatory, antioxidant, anti-fibrosis, anti-cough, anti-cancer, anti-viral, anti-arrythmia, prevention of atherosclerosis, immune regulation of fat metabolism, anti-aging, protection of liver function and Naringenin can inhibit inflammatory factors through the NF-B pathway and reduce lung inflammation induced by lipopolysaccharide (LPS) [15]. It was found that through the establishment of lipopolysaccharides-induced acute lung injury model, naringin can protect and treat lung injury by down-regulating TNF-α and other factors [16]. Formononetin is a typical phytoestrogen due to its isoflavone structure [17]. Current studies have shown that formononetin can induce tumor cell apoptosis and inhibit tumor cell proliferation in the treatment of bladder cancer [18], gastric cancer [19] and other malignant tumors. In addition, formononetin can enhance antioxidant enzyme activity and regulate energy metabolism to play an anti-fatigue role by reducing the generation of free radicals [20].

IL-6 is a multifunctional active factor, which can be induced by a variety of cells. IL-6 can stimulate the proliferation and differentiation of cells involved in immune response and improve their functions. CRP is an acute reactive protein secreted by the liver under the regulation of IL-6, and is a sensitive inflammatory response marker with immune regulatory effect. When the body is in the acute stage of infection, trauma and inflammatory lesions, CRP level rises sharply. STAT3 for signal transduction and transcriptional activation factor (STATs) is one of the members of the family, is a kind of can and target gene regulation zone cytoplasm of DNA binding protein, is responsible for the regulation of cell growth, proliferation, differentiation and apoptosis, and a series of important physiological processes, is essential in maintaining steady, especially for the damage of epithelial tissue regeneration plays an important role in [21-23]. CASP3 is a key factor that induces apoptosis. When CASP3 is activated, it ACTS on some members of the Bcl-2 family, such as Bad, thereby causing the destruction of structural proteins and DNA stability in the host cell and causing cell apoptosis [24]. As a common protein kinase of THE MAPK family, MAPK14 is expressed in inflammation-stimulated tissues and participates in the inflammatory response [25].

In this study, through the method of network pharmacology, it was found that YQAG inhibited inflammatory response, regulated immunity, and alleviated the symptoms of influenza mainly through the gene targets of IL-6, STAT3, MAPK14 and CASP3, so as to play an anti-influenza therapeutic role.

5. Conclusion

In summary, this study applied the network pharmacology

method to explore and analyze the anti-influenza action mechanism of YQAG, providing important information for further understanding of the mechanism of compound-target-disease interaction, and also providing ideas and reference basis for further research. However, there are still some deficiencies in this study. The mechanism of action of yinqiao pyretic particles is analyzed only from the theoretical level, and the prediction results cannot completely prove its correctness. Due to the incomplete development of network information technology and the lack of timely update of database, the research has some limitations. Therefore, our research group will further verify the results of this study through experiments in the future.

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