Uncovering The Mechanism of Shiwuwei Luodimingmu Wan On Cataract Via Network Pharmacology

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Abstract

Cataract is one of the most common eye diseases. The purpose of this study was to screen the active components and potential targets of Shiwuwei Luodimingmu Wan, then to explore the mechanism of Shiwuwei Luodimingmu Wan in the treatment of cataract through network pharmacology. Information about the chemical constituents and their targets of herbs in Shiwuwei Luodimingmu Wan was collected from traditional Chinese medicine system pharmacology (TCMSP) database. Cataract related genes were searched in Pubmed-Gene database and Genecards database. Then, the possible protein-protein interactions (PPIs) were derived from STRING database. Next, compound-target-diseases network was constructed using Cytoscape software. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using DAVID. Furthermore, we evaluated binding potential of key targets and compounds through molecular docking. There were 102 active components including quercetin, baicalein and kaempferol in Shiwuwei Luodimingmu Wan and 28 potential targets related to cataract. More importantly, these genes are involved in several pathways associated with cataract. Docking results showed that core compound (quercetin) had certain affinity with *MAPK14*. This study revealed that the ability of Shiwuwei Luodimingmu Wan to produce clarity of vision may was achieved through quercetin to affect multiple targets and pathways.

1. Introduction

Cataract is a common blinding eye disease [1], which has the highest rate of blinding in China. Epidemiological investigations and experimental studies have confirmed that long-term and chronic ultraviolet radiation is closely related to the occurrence of cataract [2],[3],[4],[5],[6]. There are many factors affecting the intensity of ultraviolet radiation, such as latitude, altitude, surface reflection, outdoor exposure time and occupation [7]. Tibet is a region with high altitude, low latitude and perennial snow cover [8]. Its ultraviolet radiation is very strong, so the incidence of cataract is significantly higher than that of other regions [9]. However, in recent years, due to changes in the environment [10], the prevalence of cataract and the rate of blindness outside Tibet are increasing year by year, which seriously affects the vision and life of patients [11],[12]. We know that some patients choose surgery and phacoemulsification is an important way to treat cataract [13],[14],[15]. Nevertheless, from the perspective of clinical practice, there are inevitably some postoperative complications after surgery [16]. Corneal edema is a common postoperative complication [17], and studies showed that postoperative adjustment by combining traditional Chinese medicine can improve the effect of cataract treatment [18].

Some plateau herbs have been found to be effective in treating cataract. The commonly prescribed for the treatment of cataract is Tibetan medicine - Shiwuwei Luodimingmu Wan, composed of Luodi, *Gypsum Rubrum* (Hanshuishi), *Carum carvi* (Zanghuixiang), Shihuihua, *Glycyrrhiza uralensis Fisch* (Gancao), *Chebulae Fructus* (Hezi), Zhaxungao, Caryophylli Flos (Dingxiang), Jinqianbaihuashe, *PhyllanthiFructus* (Yuganzi), Tiexie, *Hematite* (Daizheshi), *Carthami Flos* (Honghua), Maohezi and Lvronghao. It has the function of clearing liver and improving eyesight. In the prescription, Luodi plays a major role in the treatment of cataract. Luodi hydrolyzed by proteolytic enzyme can dissolve and

dissipate the denaturetic protein and make the crystal transparent; Zanghuixiang, Gancao, Hezi, Lvronghao have anti-inflammatory and anti-oxidation effects [19], so the crystal damaged by ultraviolet radiation can be repaired in time; Honghua can dilate blood vessels and improves circulation [20]; In addition, Jinqianbaihuashe provides protein and fat nutrition, which can promote blood circulation and remove blood stasis, as well as disperse and open knots. The combination of various drugs can effectively improve eye circulation, provide crystal nutrition, effectively dissolve crystal denaturated protein, and restore crystal transparency. However, the molecular mechanism of Shiwuwei Luodimingmu Wan in treating cataract remains unclear. Network pharmacology is a comprehensive research method integrating chemical informatics, bioinformatics, network biology and traditional pharmacology [21], which can systematically and comprehensively reveal the bioactive components and the mechanism of action of traditional Chinese medicine prescriptions, and reflect the relationship between multiple components and multiple targets of traditional Chinese medicine [22]. Therefore, we used the research method of network pharmacology to explore the mechanism of Shiwuwei Luodimingmu Wan in the treatment of cataract, and used molecular docking technology to simulate and predict the interaction of disease-related targets and related components.

2. Material And Methods

2.1 Screening of active compounds in herbs

We found that Shiwuwei Luodimingmu Wan is a prescription drug for treating cataract on the reputable Tibetan medicine website (http://www.xyzyw.cn/). Chemical compositions of herbs in Shiwuwei Luodimingmu Wan were collected from TCMSP database. Then the constituents were filtered by integrating Oral bioavailability (OB) and drug-likeness (DL), and chemical components that cannot satisfy both OB ≥ 30% and DL ≥ 0.18 were deleted as suggested by the TCMSP database [23].

2.2 Identification of target genes associated with compounds

The corresponding target proteins were found according to the compounds from TCMSP. Then, the multiple proteins were converted into corresponding genes using STRING (https://string-db.org/cgi/input.pl) database with the “Homo sapiens” species selecting.

2.3 Potential target genes for cataract

In Pubmed-Gene (https://www.ncbi.nlm.nih.gov/gene/?Term) and Genecards (https://www.genecards.org/) database, inputting "cataract" to lookup cataract related genes.

2.4 Protein–protein interactions network

The target genes of the compounds were mapped to the cataract genes, and the common targets were collected in Venny2.1.0. After that, Protein–protein interactions (PPIs) were derived from STRING

database. Then, the network was built by Cytoscape software, and the core targets were selected by degree values.

2.5 Construction of compounds-targets network

To understand the potential effect of Shiwuwei Luodimingmu Wan in the treatment of cataract, compounds-targets network was performed using Cytoscape software.

2.6 Function and pathway enrichment analysis

In order to explore the signal transduction pathway and function of targets, we carried out GO function analysis and KEGG pathway enrichment analysis by using DAVID (https://david.ncifcrf.gov/). R-package software was used to compare and verify the retrieval results with DAVID database, and the visualization was performed by using bubble graph and bar graph. Key pathways and their associated genes and components were analyzed using Cytoscape software to know the mechanism of action of Shiwuwei Luodimingmu Wan for curing cataract.

2.7 Molecular docking

Molecular docking is performed to simulate the action mode and intensity of potential drug and target in the treatment of diseases [24].According to the number of nodes, the proteins and chemical components of the docking were screened. The 3D structures of candidate protein targets were downloaded from the SWISS-MODEL (https://swissmodel.expasy.org/interactive). The structures of predicted monomer small molecular were obtained from the ChemSpider database (https://www.chemspider.com/). Then AutoDockTools-1.5.6 was applied to not only remove water molecules and pro-ligand small molecules, but also hydrogenate and charge [25]. Finally, molecular docking calculations were performed using Autodock Vina 1.1.2 and docking results were analyzed by Discovery Studio 2016 Client.

3. Results

3.1 Active ingredients filtering

A total of 157 components of seven herbs in Shiwuwei Luodimingmu Wan were extracted from TCMSP, including 1 ingredient in Luodi, 92 in Gancao, 8 in Hezi, 6 in Dingxiang, 18 in Yuganzi, 22 in Honghua and 10 in Lvronghao (Fig. 1). Due to duplication, fourteen of these compounds were deleted, resulting in 143 effective active ingredients. Basic information of these constituents in Shiwuwei Luodimingmu Wan is listed in **Supplementary Table 1**. We found that Gancao had the most chemical constituents among the herbs. And among other ingredients, quercetin is found in six other herbs except Hezi.

3.2 The main therapeutic targets predicted

In pubmed-gene and genecards databases, 221 and 3,712 disease-related genes were found, respectively. We finally collected a total of 219 coincidence genes by taking intersection, and 28 genes were cross-

identified with 331 drug target genes (Fig. 2). The target names and gene symbols of these 28 genes are shown in **Supplementary Table 2**.

3.3 PPIs network analysis

There are 27 interacting targets in the network (*PDE10A* are not involved in protein interaction). The darker the color, the more proteins it interacts with. The darkest is *TP53*, then *VEGFA*, *MAPK14*, *ESR1*, *SOD1*, *CAT* (Fig. 3). These genes are functionally classified into oxidase, cytogenic, protease, and apoptotic inhibitors. They may play a key role in the network. The top ten genes are presented in **Supplementary Table 3** according to the degree values.

3.4 Compounds-targets network analysis

As you can see from Fig. 4, we found that different active ingredients could act on the same targets and on different targets, which fully reflected the mechanism of action of the multi-component and multi-target of the Tibetan medicine Shiwuwei Luodimingmu Wan. According to the relatively high number of nodes, three small molecules were screened and corresponding targets were found.

3.5 Analysis of gene functions and target pathway

GO enrichment analysis in DAVID resulted in 148 GO items ($P < 0.05$), among which 105 were biological process (BP) items, 16 were cellular component (CC) items, and 27 were molecular function (MF) items, accounting for 71%, 11% and 18% respectively. We listed the top 10 items in each category, as shown in Fig. 5. Biological processes involved cell aging, cellular response to hypoxia, negative regulation of cell growth, positive regulation of gene expression, positive regulation of peptidyl-tyrosine phosphorylation, etc. Cellular component involved extracellular space, nuclear chromatin, cytoplasm, cytosol, peroxisome, mitochondrion, etc. Molecular function involved enzyme binding, identical protein binding, transcription factor binding, protein homodimerization activity, RNA polymerase II transcription factor activity, etc.

According to the enrichment analysis of KEGG pathways by DAVID, 20 signaling pathways were screened with $P < 0.05$. We showed the top 20 KEGG pathways in Fig. 6. The schematic diagram of the path is shown in Fig. 7(TOP 5). Analysis of the targets-pathways network (Fig. 8) revealed that *TP53*, *BCL2* and *MAPK14* are involved in a large amount of cataract-related pathways.

3.6 Molecular docking

Molecular docking analysis provided a visual explanation of the interaction between key component and protein targets associated with contract. Here, molecular docking for Shiwuwei Luodimingmu Wan ingredient (quercetin) and *TP53*, *BCL2* and *MAPK14* proteins were analyzed, and the results showed that

quercetin had strong affinity with *MAPK14* proteins (Fig. 9). We found Van der Waals force and π-π stacking were the main forms of interaction.

4. Discussion

In this study, based on the network pharmacology method, we identify bioactive compounds, potential targets and the pathways modulated by these compounds in Shiwuwei Luodimingmu Wan treatment of cataract. 102 active components and 28 key targets were confirmed. Interestingly, we found that quercetin has relatively high number of nodes and the number of related genes was also relatively more. This observation indicated that quercetin is a key ingredient in the prescription for treating cataracts. Quercetin is a flavonoid with anti-oxidation, anti-viral and antibacterial effect, and can inhabit inflammatory cells activation. Previous studies have suggested that quercetin involve in the prevention of cataract. The involved mechanisms in these findings include mitigating the production of reactive oxygen species, inhibiting vascular endothelial growth factor pathways, suppressing tumor suppressor gene and apoptosis, and suppressing the production of inflammatory markers [26–29].

We further focused our attention on the target genes and related pathways. Functional enrichment analysis predicted multiple cataract-related pathways. These pathways are closely associated with apoptosis and senescence and the occurrence of cataract is related to lens epithelial cell apoptosis, nutrition and oxidation [30, 31]. The observation is in agreement with the reported. Moreover, according to the comprehensive analysis of PPI, compound-target network and pathway-target network, *TP53*, *BCL2* and *MAPK14* was screened out, which may participate in integrated regulation of Shiwuwei Luodimingmu Wan through protein interactions and cataract-related pathways. In human genes, *TP53* is a famous tumor suppressor gene, which plays an important regulator of cell growth, proliferation damage repair [32],[33],[34]. The most prominent feature of p53 is that it is a transcription factor, which affects the occurrence and development of diseases by targeting many genes and pathways related to apoptosis or cell cycle regulation. Another key target, Apoptosis regulator Bcl-2 transcribed by the *BCL2*, regulated mitochondrial outer membrane permeability, then activated downstream caspase cascades for apoptosis and inhibits p53-mediated apoptosis [35]. It is reported that the apoptosis of lens epithelial cells was negatively regulated by *BCL2* [36]. It is noteworthy that *MAPK14* is the most highlight targeted gene in prescription-disease interactions. The protein encoded by this gene is a member of the MAP kinase family, as integrators of multiple biochemical signals, involved in cell proliferation, differentiation, transcriptional regulation and development. To further analysis the interactions between quercetin with *TP53*, *BCL2* and *MAPK14*, we performed the molecular docking, and suggested the effective bindings and stable complex with low energy between the ligand and receptors. Vina score indicated strongest relationship between quercetin and *MAPK14*.

Out of all the analysis, *MAPK14* seems to be the most interesting target. The effect of quercetin on key genes *MAPK14* may be a crucial factor of Shiwuwei Luodimingmu Wan in the treatment of cataract. Nevertheless, the specific mechanism has not been studied, and needs to be verified in combination with experiments such as mouse models.

5. Conclusions

In summary, this study first preliminarily verified the pharmacological mechanism of Shiwuwei Luodimingmu Wan in the treatment of cataract by using network pharmacology and molecular docking, which laid a good foundation for the follow up in depth discussion. We hope it will be helpful for the development of new drugs and the treatment of cataract.

Abbreviations

SLW: Shiwuwei Luodimingmu Wan; TCMSP: Traditional Chinese Medicine Systems Pharmacology; PPIs: protein-protein interactions; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; OB: Oral bioavailability; DL: drug-likeness; BP: biological process; CC: cellular component; MF: molecular function.

Declarations

Acknowledgements

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Statement of Ethics

The paper is exempt from ethical committee approval. TCGA and GEO belong to public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues and other conflicts of interest.

Author Contributions

Zhanhao Zhang and Tianbo Jin designed the study and wrote the manuscript. Yuliang Wang conducted component analysis. Hongyan Lu、Yongjun He、Li Wang、Jianwen Zheng offered some advices on this research and handled the figure. All authors have reviewed and approved the final manuscript.

Availability of data and materials

All relevant data are within the manuscript.

Consent for publication

Not applicable

Conflicts of Interest

There are no conflicts of interest to declare.

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Figures

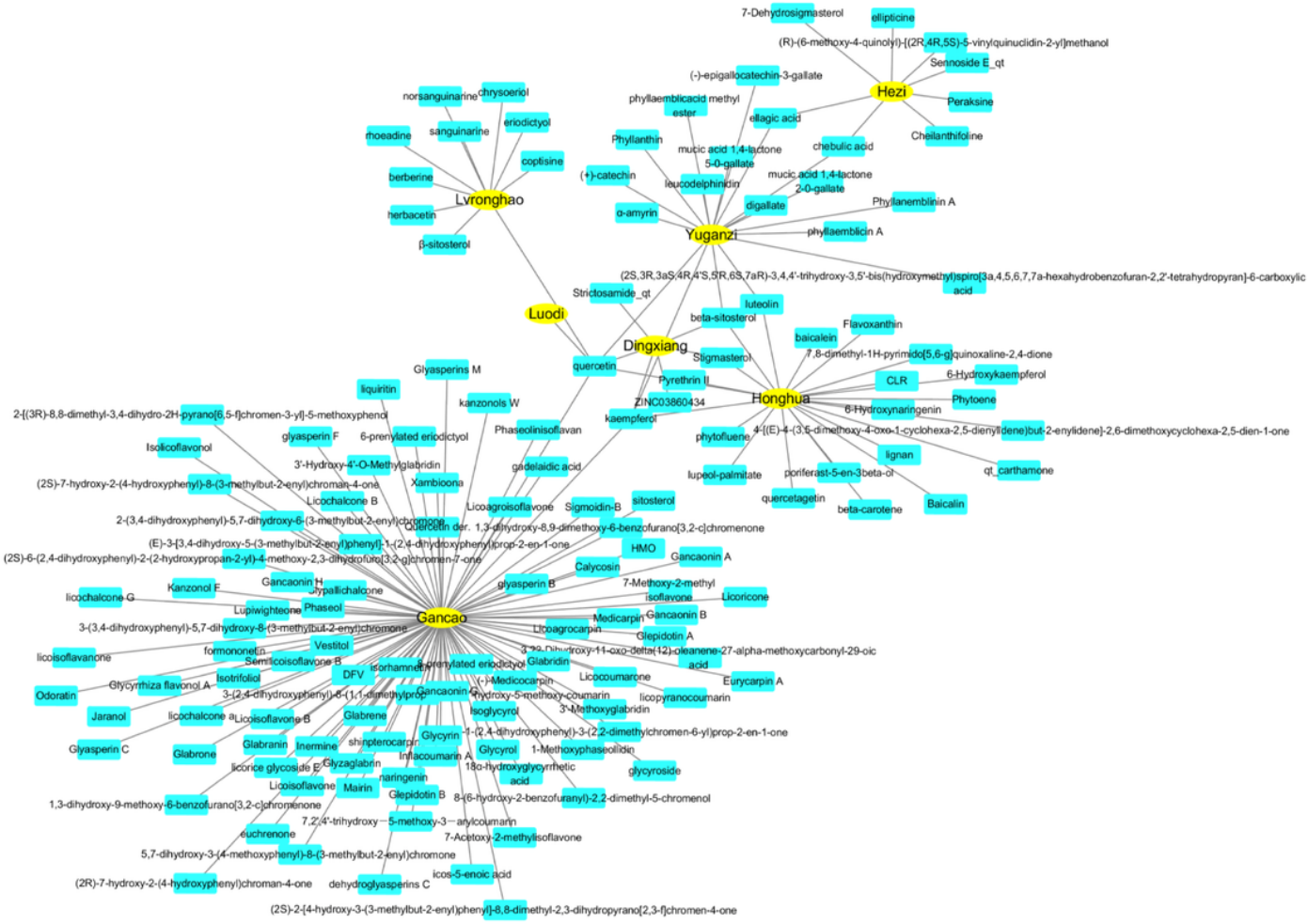

Figure 1

Shiwuwei Luodimingmu Wan herbs-molecules network.

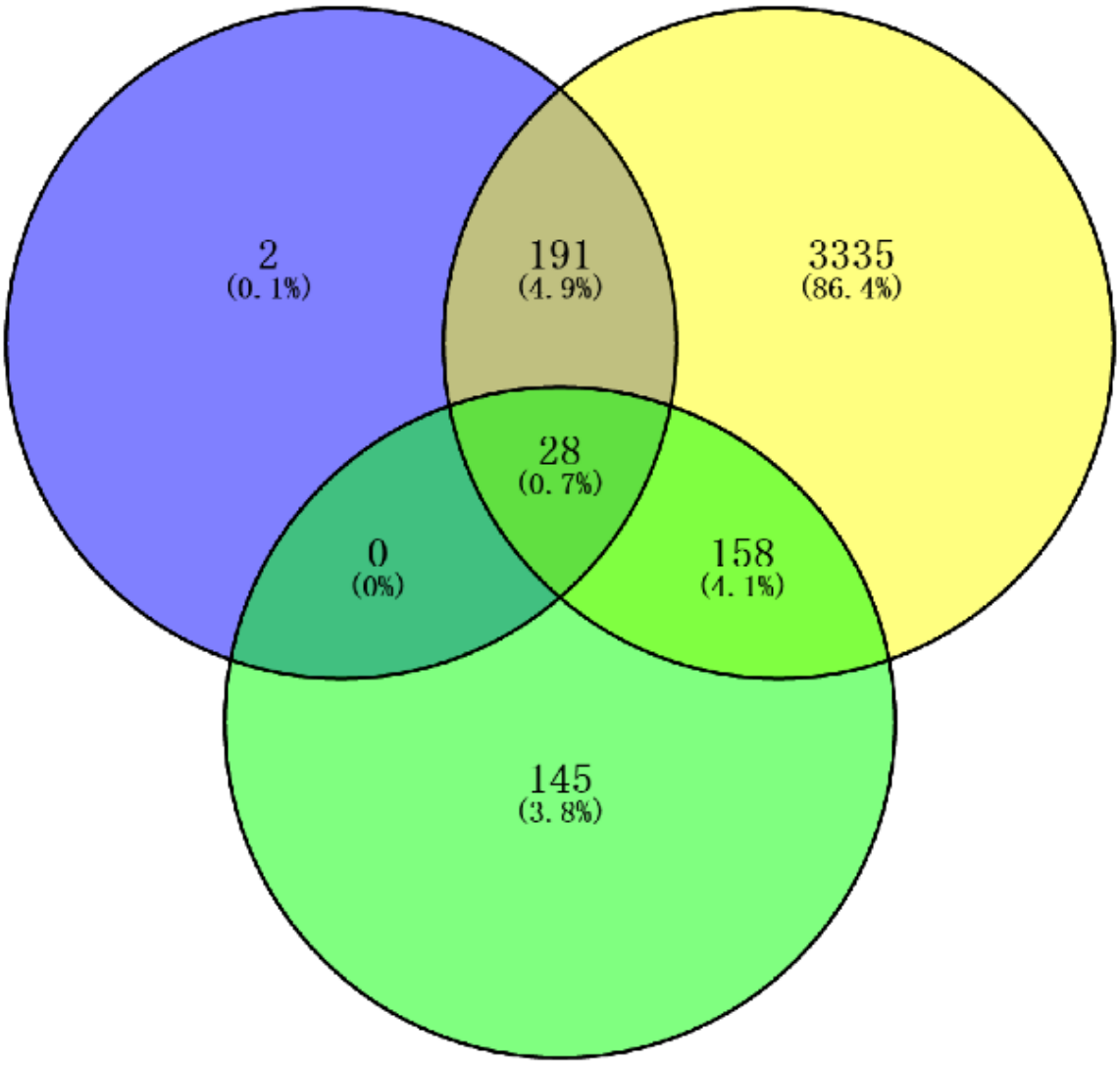

Figure 2

Venn diagram of the relationship between the targets of Shiwuwei Luodimingmu Wan and the cataract related targets.

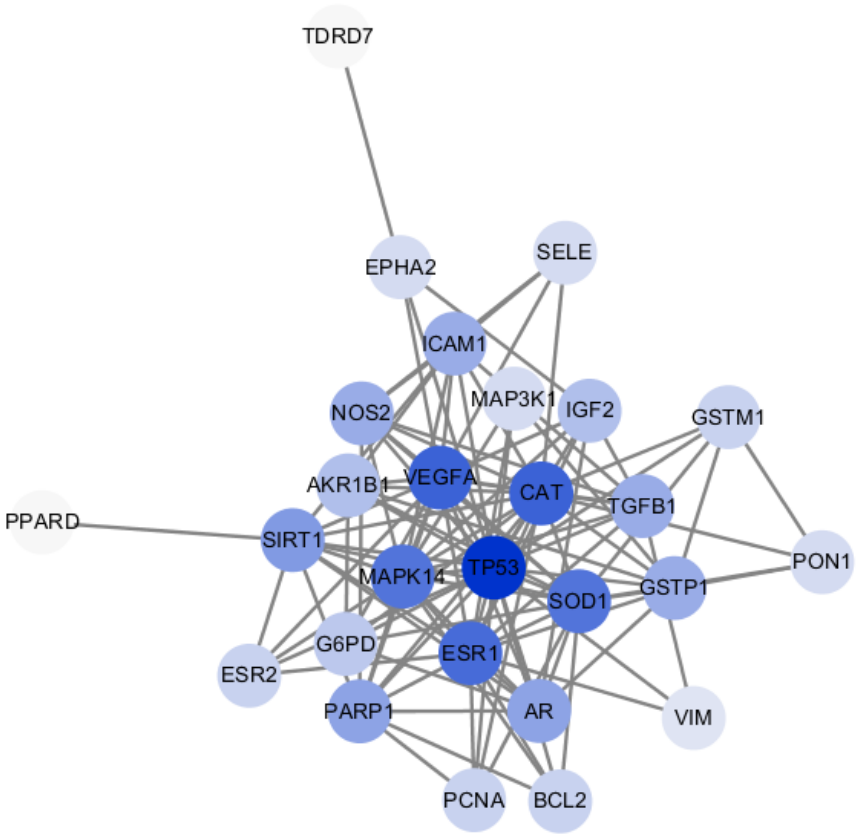

Figure 3

Protein -protein interactions（PPIs）network.

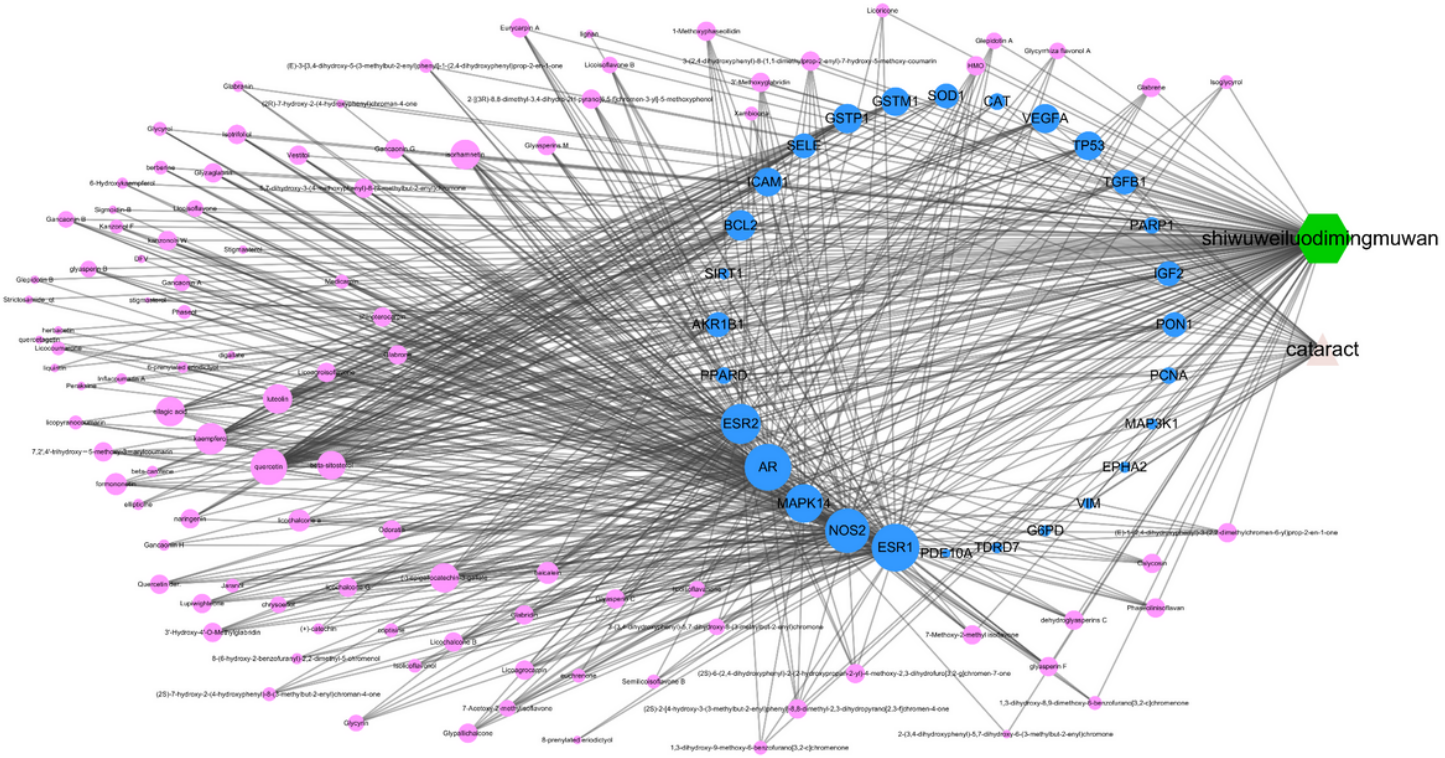

Figure 4

Potential active compounds-targets network of Shiwuwei Luodimingmu Wan acting on cataract.

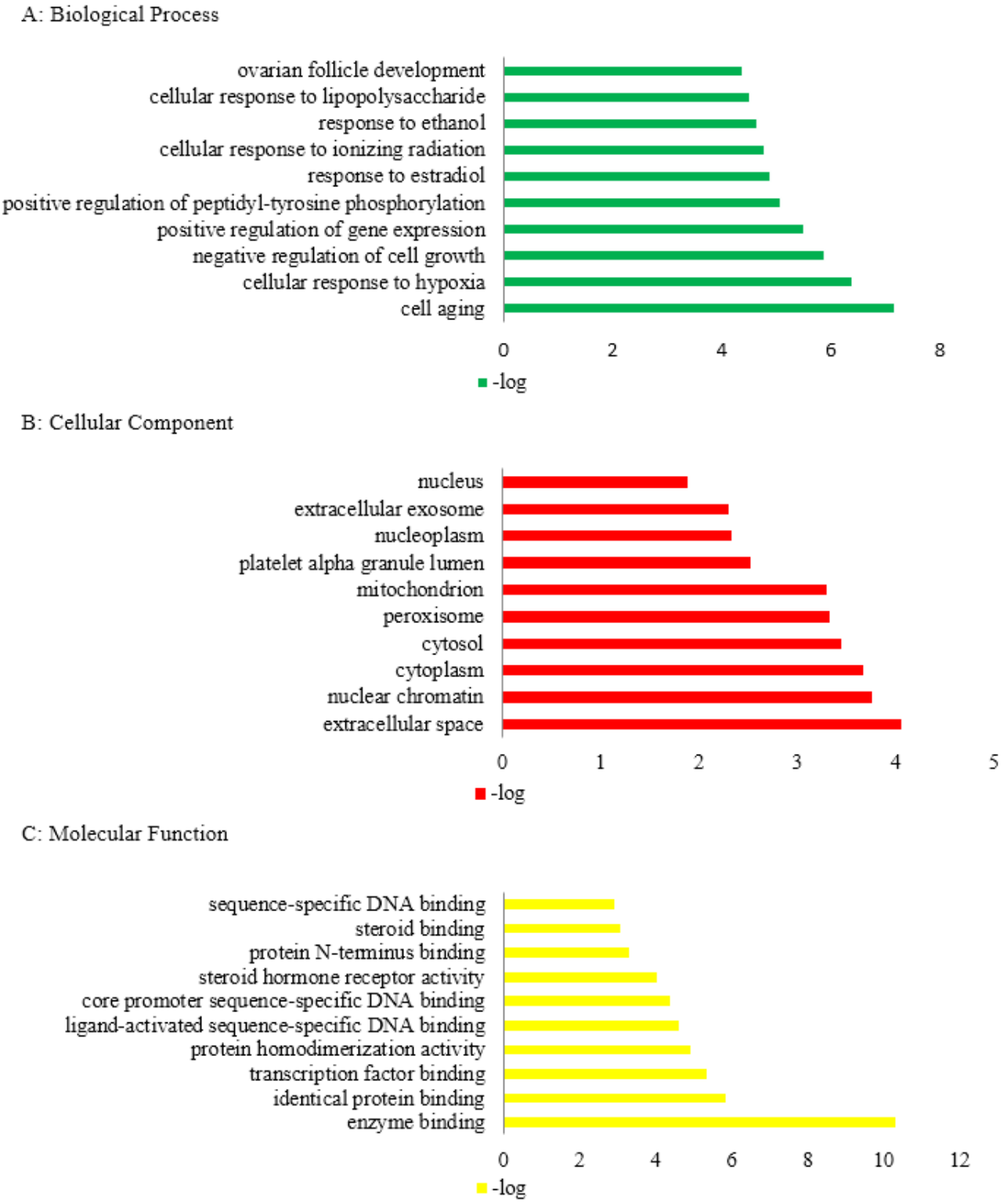

Figure 5

Gene Ontology (GO) analyses of major genes.

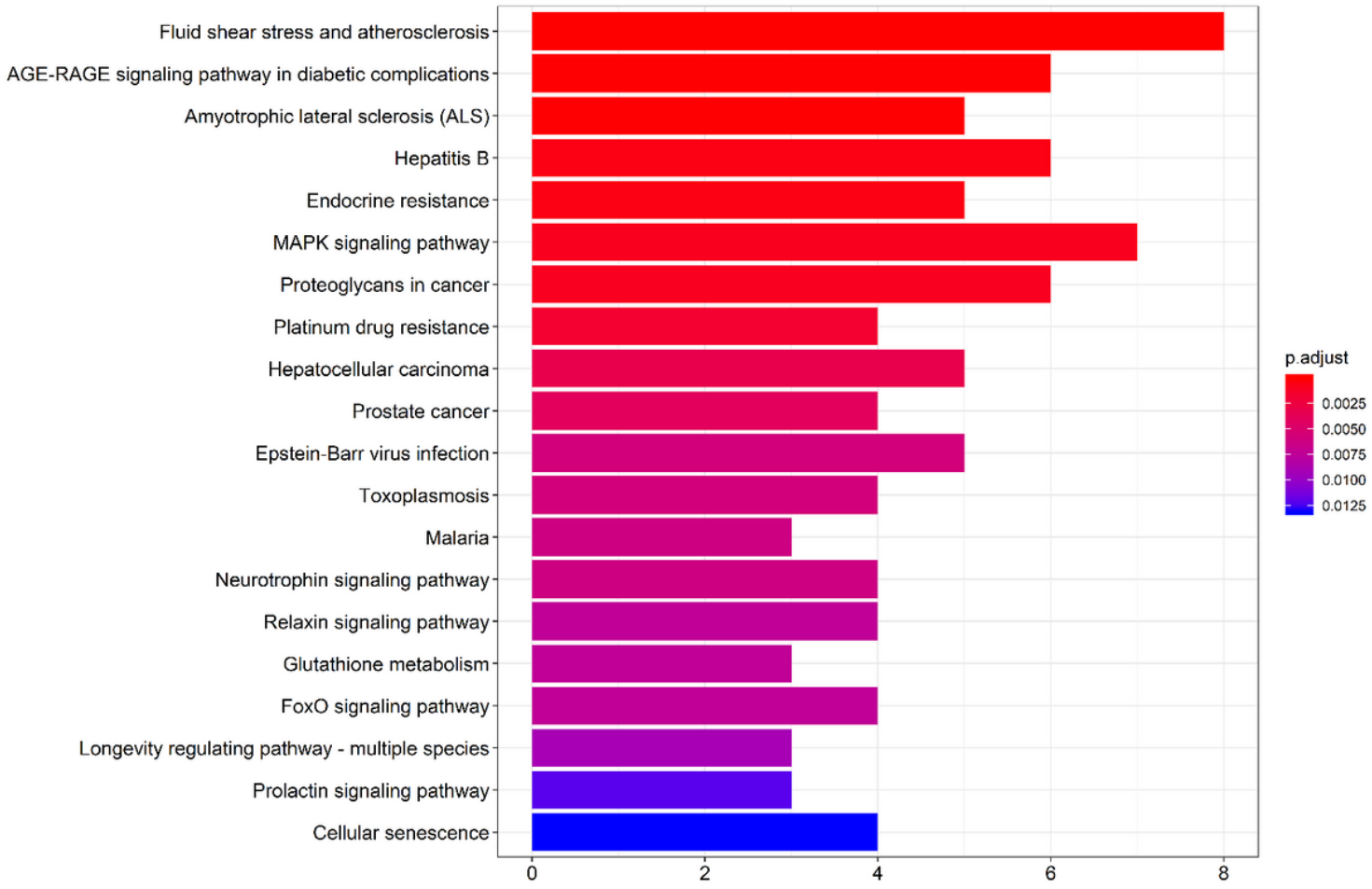

Figure 6

KEGG enrichment analysis diagram.

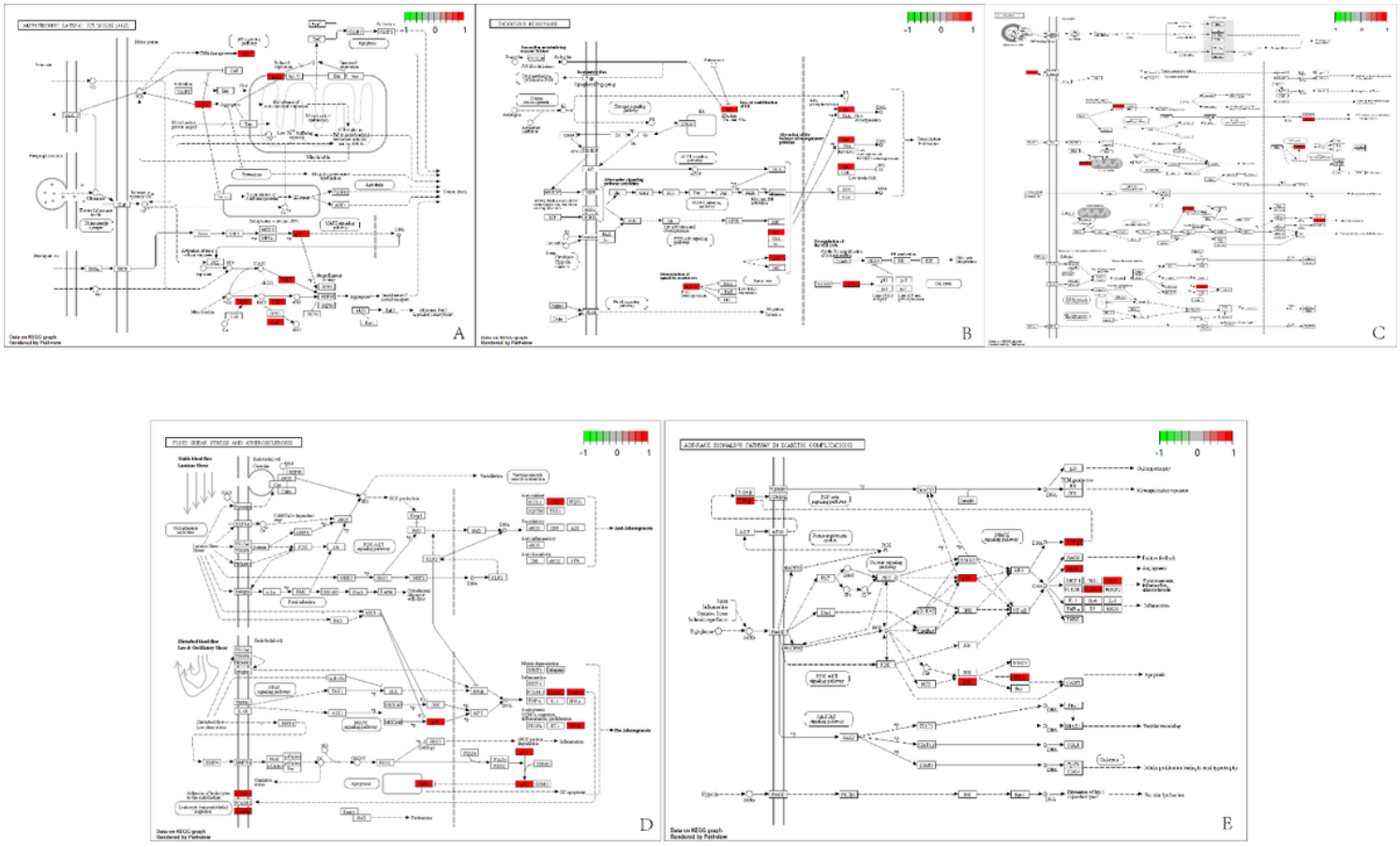

Figure 7

Diagram of disease-related signaling pathways.

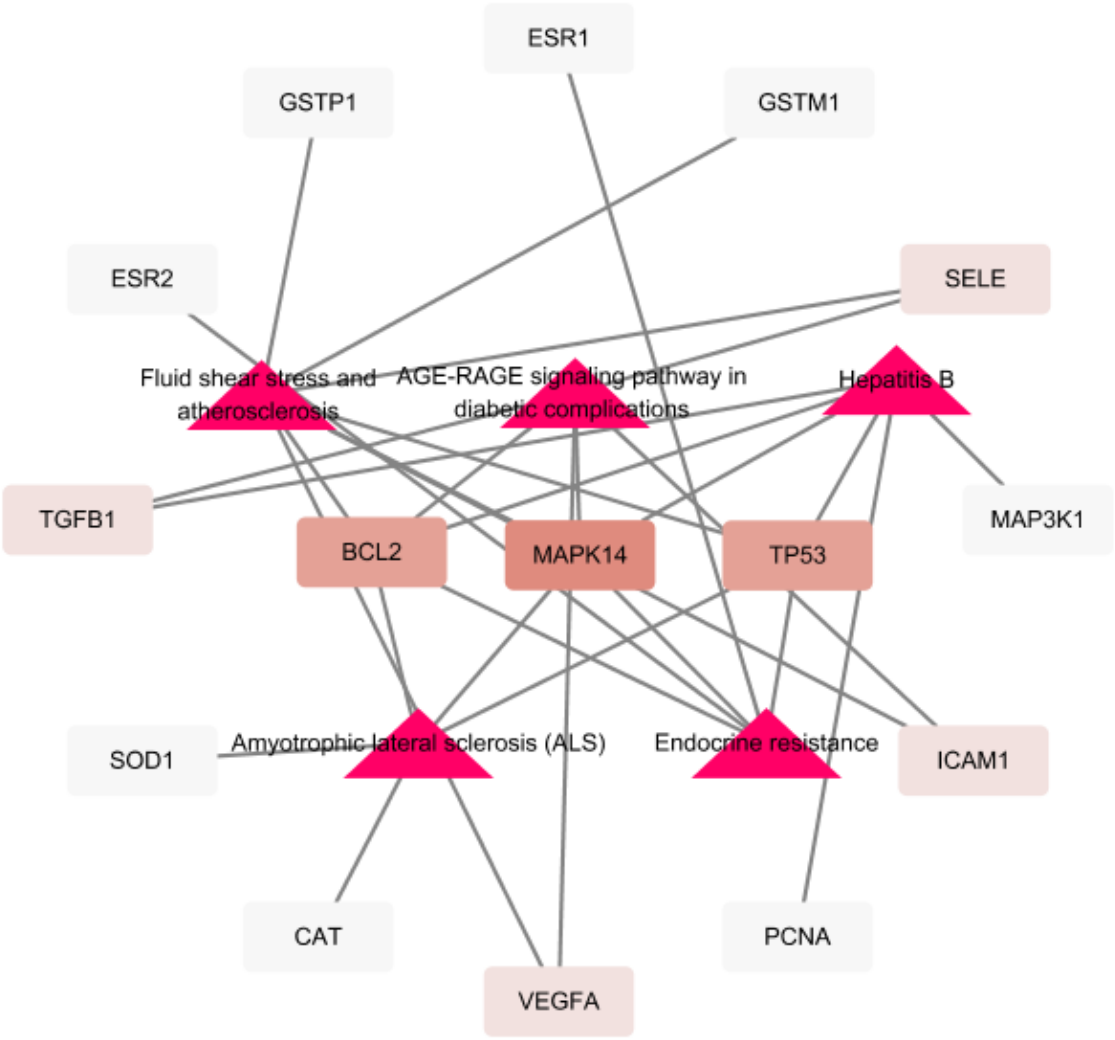

Figure 8

Targets-pathways network.

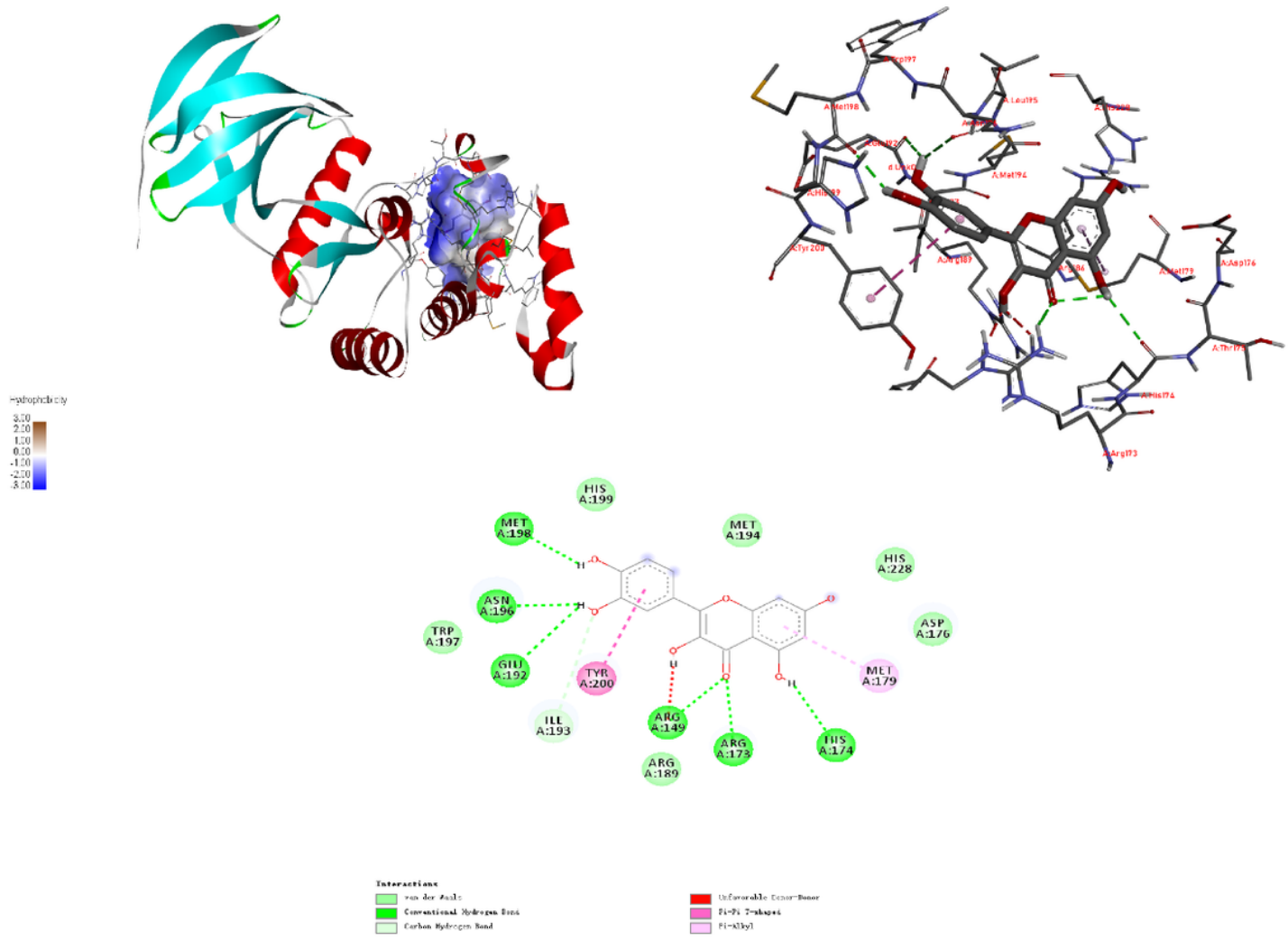

Figure 9

The analysis of the interaction between quercetin and MAPK14.

Supplementary Files

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- SupplementaryTables.docx