Exploring a novel traditional Chinese medicine prescription containing *Chrysanthemi Flos* for retinal diseases: an integrated strategy of data mining and network pharmacology

Jin-Xue Zhang1,2, Kai-Lun Zhang3, Xiang-Wei Chang1,3,4,5,6,7, Jun-Fei Gu8, Shi-Bing Wei1, Bo-Yang Zhu1, Qi Wang1,3,4,5, De-Ling Wu2,7, Shuang-Ying Gu1,3,4,5, Hui Yan4, Jin-Ao Duan1

1College of Pharmacy, Anhui University of Chinese Medicine, Hefei 230012, China. 2MOE-Anhui Joint Collaborative Innovation Center for Quality Improvement of Anhui Genuine Chinese Medicinal Materials, Hefei 230012, China. 3Institute of Pharmaceutics, Anhui Academy of Chinese Medicine, Hefei 230012, China. 4Anhui Provincial Key Laboratory of Pharmaceutical Preparation Technology and Application, Hefei 230012, China. 5Engineering Technology Research Center of Modernized Pharmaceutics, Anhui Education Department [AUCM], Hefei 230012, China. 6Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Nanjing University of Chinese Medicine, Nanjing 210023, China.

*These authors contributed equally to this work and are co-first authors for this paper.

**Correspondence to:** Xiang-Wei Chang, College of Pharmacy, Anhui University of Chinese Medicine, No. 350, Longzhuzhi Road, Xinhua District, Hefei 230012, China. E-mail: cxhweing@163.com. Jun-Fei Gu, Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Nanjing University of Chinese Medicine, No. 138, Xianlin Avenue, Xianlin University City, Nanjing 210023, China. E-mail: gujunfei@njucm.edu.cn.

**Author contributions**
Zhang JX, Chang XW and Gu JF conceived the project. Zhang JX, Zhang KL, Wang Q and Zhu BY filtered and downloaded data. Zhang KL, Wei SB, Wu DL and Gu SY analyzed the data. Zhang JX, Zhang KL and Chang XW drafted the manuscript. Chang XW, Gu JF, Yan H and Duan JX were responsible for data verification, supervision and reviewed the manuscript. All authors read and approved the final manuscript.

**Competing interests**
The authors declare no conflicts of interest.

**Acknowledgments**
This work was supported by the National Natural Science Foundation of China (Grant No. 82104701), Science Fund Program for Outstanding Young Scholars in Universities of Anhui Province (Grant No. 2022AH030064), Key Project at Central Government Level: The Ability Establishment of Sustainable Use for Valuable Chinese Medicine Resources (Grant No.20060302), Youth Project of Natural Science Foundation of Anhui Province (Grant No. 2208085SH268), Key Project of Natural Science Research in Universities of Anhui Province (Grant No. KJ2020A0430, KJ2021A0544), Foundation of Anhui Province Key Laboratory of Pharmaceutical Preparation Technology and Application (Grant No. 2021KFXT10), China Agriculture Research System of MOF and MARRA [Grant No. CARS-21], Talent Support Program of Anhui University of Chinese Medicine (Grant No. 2020czyk007).

**Peer review information**
Traditional Medicine Research thanks all anonymous reviewers for their contribution to the peer review of this paper.

**Abbreviations**
TCM, traditional Chinese medicine; GLA, glaucoma; DR, diabetic retinopathy; AMD, age-related macular degeneration; MD, molecular dynamics; RMSD, root mean square deviation; RMSF, root mean square fluctuation; Rs, radius of gyration; RGG, retinal ganglion; RPE, retinal pigment epithelium; PPI, protein-protein interaction; BP, biological process; CC, cellular component; MF, molecular function; VEGF, vascular endothelial growth factor; GO, gene ontology.

**Citation**
Highlights
An integrated strategy of data mining, network pharmacology, molecular docking and molecular dynamics simulation was applied to screen a novel traditional Chinese medicine (TCM) prescription against retinal diseases from patents containing *Chrysanthemi Flos* and explore its medication rule and mechanism.

Medical history of objective
*Chrysanthemi Flos* is a medicinal and edible cognate. Its therapeutic uses were first chronicled in the *Shen Nong Ben Cao Jing* (*Shen-Nong’s Herbal Classic*; 25–220 C.E.). In ancient times, *Chrysanthemi Flos* was commonly used to treat the common cold, headaches, swollen eyes, and impaired vision. Modern pharmacological studies have shown that *Chrysanthemi Flos* has broad bioactivities such as anti-inflammatory, antimicrobial, antioxidative, antiangiogenic, antihypertensive, anti diabetic, anticancer and neuroprotective effects.

Background
Human brain can get the external information approximately beyond 70% of the total through eyes, so eyes are the vital sensory organ in human body [1]. Retina is a multilayered tissue located in the posterior segment of the eyeball, closely connect with the vitreous humor and the retinal pigment epithelium (RPE) [2]. Given changes in lifestyle and the environment, the number of cases of retinal diseases is increasing, like glaucoma (GLA), diabetic retinopathy (DR), age-related macular degeneration (AMD) and branch retinal vein occlusion causing serious disruptions to daily life [3, 4]. Therefore, effective and informed eye treatment is gaining importance. Current primary treatment strategies for retinal diseases include intravitreal injection of drugs against the vascular endothelial growth factor (VEGF) drugs, vitrectomy and retinal laser photocoagulation [5–7]. Nevertheless, these therapies may lead to inafult effects that including excessive angiogenesis, increased intraocular tension and bleeding of retinal [8, 9]. Therefore, the development of effective therapeutic strategies with fewer side effects are required for curing retinal diseases.

Traditional Chinese medicine (TCM) own huge advantage in ameliorating homeostasis of the body’s internal environment, with minimal toxic side effects and good effectiveness [10]. The nature of multi-component and multi-target of TCM offers unique advantages for the treatment of retinal diseases. Accumulating evidence suggests that TCMs contribute extremely to the treatment of retinal diseases through impacting on oxidative stress, angiogenesis and inflammatory processes [11, 12]. Panax notoginseng saponins alleviate DR by inhibiting retinal inflammation [13]. *Dendrobium chrysotoxum* Lindl. exerts therapeutic effects against DR by alleviating retinal angiogenesis and ameliorating retinal inflammation [14]. *Chrysanthemi Flos* is a typical traditional herbal medicine for brightening eyes widely used for thousands of years [15]. Traditional and modern studies have shown that TCM prescriptions containing *Chrysanthemi Flos*, including *Mingmushiaoyao* granules (composed of *Chrysanthemi Flos*, *Lycii Fructus*, *Bupleuri Radix*, *Angelicae Sinensis Radix*, *Paoniae Radix Alba*, *Glycyrrhizae Radix et Rhizoma*, *Poria*, *Atractylodis Macrocephalae Rhizome*, *Acori Tatarinowii Rhizome and Moutan Cortex*) and *Qijudihuang* pill (composed of *Lycii Fructus*, *Chrysanthemi Flos*, *Corni Fructus*, *Moutan Cortex*, *Discoreae Rhizoma*, *Poria*, *Alismatis Rhizoma* and *Rehmanniae Radix Praeparata*), have good curative effects on retinal diseases [16, 17]. Therefore, TCM prescriptions containing *Chrysanthemi Flos* have application prospects and development potential in the therapy of retinal diseases.

As we know, patent is the indicator and promoter of technological innovation and industry development [18]. There may contain various hidden prescription rules that are valuable to clinical practice. As a widely applied technology in intelligence data handling, data mining facilitates researchers to sniff out potential medication rule through multidimensional analysis of prescriptions [19]. So, digging related information and medication rules from abundant patents is a precious way to provide reference for development of new drug. Network pharmacology is a novel approach combined systems biology, pharmacology and computer technology to study the network relationships between the active component of TCM prescriptions and corresponding targets, metabolic pathways and diseases [20]. This approach is in accordance with the emphasis of TCM in holistic theory for the therapy of diseases with multiple ingredients and multiple targets. Molecular docking is a simulation method in theoretical level and is used to predict binding effect between receptors and molecule ligands widely used to corroborate results derived from network pharmacology. Molecular dynamics (MD) simulation is a computer-based technology that can be used to simulate small molecules movement in the body. In recent years, network pharmacology combined with molecular docking and MD simulation technology has been used to study underlying treatment mechanism of TCM [21–23]. Sun et al. used network pharmacology, molecular docking, and MD simulation to investigate mechanism of ischemia-reperfusion injury in spinal cord [21]. Wang’s team employed network pharmacology, molecular docking, and MD simulation approaches to elucidate the mechanism of Qingfeiyin in the therapy of acute lung injury [22]. However, to the best of our knowledge, there have been no reports on exploration of the mechanism of TCM prescriptions containing *Chrysanthemi Flos* for therapy of retinal diseases based on network pharmacology and molecular docking strategy.

Herein, an integrated strategy of data mining, network pharmacology, molecular docking and MD simulation was applied to screen a novel TCM prescription against retinal diseases from patents containing *Chrysanthemi Flos* and explore its medication rule and molecular mechanism. Firstly, TCM prescriptions containing *Chrysanthemi Flos* used for treatment of various retinal diseases were collected by searching patent databases. A novel TCM prescription was then screened based on association rule analysis. Next, a network of core TCMs-core active ingredients-core targets-core pathways was constructed to clarify the internal mechanisms of the novel TCM prescription in the treatment of retinal diseases using network pharmacology. Molecular docking and MD simulation technology were further used to validate the results derived from network pharmacology. This study further provides a perspective on understanding the molecular mechanism of the novel TCM prescription for treating retinal diseases and draws a direction for developing new drugs against retinal diseases. The study flowchart is presented in Figure 1.

Materials and methods

Data sources
The National Intellectual Property Administration of PRC (https://ps-system.cponline.cnipa.gov.cn/), PatSnap Patent Database (https://www.zhihuixi.com/) and Qizhidaos Patent Database (https://patents.qizhidaos.com/) were used as data sources. Both computer retrieval and manual reading tests were performed using the terms below to search by title, abstract, and claim of patents: “Chrysanthemi Flos” and “retina”. A comprehensive search of patents worldwide was performed from the date of database inception publication date up to June 1, 2022. The retrieval results were assessed individually and manually screened for all related patents in the database. Finally, prescriptions were analyzed based on principles of the inclusion and exclusion stated below.

Inclusion and exclusion criteria
Inclusion rules were TCM prescriptions containing *Chrysanthemi Flos* for the treatment of retinal diseases with clear provenance, composition and dosage. Patents were excluded if they focused on (1) prescriptions for retinal diseases with only a single TCM; (2)
prescriptions for retinal diseases with western medicine; (3) detection for retinal diseases; (4) food, beverage and health products related to retinal diseases; (5) prescriptions for retinal diseases with deficient composition and dosage.

Prescriptions input and TCM name specification
Names of the TCMs in prescriptions were normalized from the People’s Republic of China Pharmacopoeia, Encyclopedia of Traditional Chinese Medicinal Substances and the textbook of Traditional Chinese Medicine (the 13th five-year Plan of higher Education version). The ethnic medicines and conventional utilization of medicines were named standardly and inputted into Microsoft Office Excel 2019 software, for example, “Danpi” was uniformly standardized as “Moutan Cortex”. The same TCM whose medicinal efficacy changes significantly due to different processing methods was entered separately, such as “Polygoni Multiflori Radix” and “Polygoni Multiflori Radix Praepapata”. If the prescription patents contained TCM extracts, the corresponding Chinese medicines were entered. Data for the chosen and normalized prescriptions were entered into the Excel. Two authors took charge of inputting data individually and earnestly to guarantee the accuracy of mining data.

Data analysis

Figure 1 Flow diagram of this study. TCM, traditional Chinese medicine; GLA, glaucoma; DR, diabetic retinopathy; AMD, age-related macular degeneration; PPI, protein-protein interaction; GO, gene ontology.
Analysis of overall evolution trend of TCM prescription patents containing *Chrysanthemi Flos* against retinal diseases. The initial extracted data from each patent included temporal distribution, spatial distribution, types of applicants and distribution of dosage forms for TCM prescription patents as well as types of retinal diseases treated by TCM prescriptions collected from patent databases containing *Chrysanthemi Flos*. Analysis of the mediation rule of TCM prescriptions containing *Chrysanthemi Flos* against retinal diseases. All data were summarized in Microsoft Excel 2019, thus establishing a database of retinal diseases treated with TCM. Statistical analyses were conducted on the frequency, the classification of TCM efficacy, four natures, five tastes, and meridian tropisms of TCMS. SPSS Statistics 27 software was applied for modeling, and the apriori algorithm was used to analyze the association rule of the above TCMS. The parameters were set as the minimum support greater than or equal to 10%, the lift greater than 1, minimum confidence at 100% and the maximum preceding item at two. When the strongly linked was set to 35 and weak link was set to 15, the closely related TCMS cluster was obtained using SPSS modeler 18.0. and Cytoscape 3.7.1 software to construct the core network of TCMS, and the degree of nodes in the network was calculated by the network analysis function. The network topological parameters of TCMS were analyzed to screen five core TCMS with highest degree as a novel TCM prescription for the therapy of retinal diseases.

**Analysis of the molecular mechanism of the novel TCM prescription against retinal diseases.** The Traditional Chinese Medicine System Pharmacology Database (TCMSP, https://tcmspw.com/tcmsp.php) was first applied to identify the active chemical compositions of a single TCM in the novel TCM prescription. Oral bioavailability and drug likeness were applied to filter active compounds. In the TCMSP database, if screening compounds oral bioavailability ≤ 30% and drug likeness ≥ 0.18, they were defined as active constituents. In conjunction with the active ingredients documented in the literature, the underlying active ingredients of the novel TCM prescription for the therapy of retinal diseases were preliminarily determined. The targets of potential active ingredients were obtained using the SwissTargetPrediction database (http://www.swistargetprediction.ch/), SEA database (https://sea.blklab.org/) and PharmMapper database (http://lilab-eust.cn/pharmmapper/). Duplicate targets were excluded. In the second step, TTD database (https://db.idrblab.net/ttd/), DrugBank database (https://go.drugbank.com/), OMIM database (https://www.omim.org/) and DisGeNET database (https://www.disgenet.org/) were used to collect disease targets by searching the keywords “glaucoma”, “diabetes retinopathy” and “age-related macular degeneration”, and the Uniprot database (https://www.uniprot.org/) was also adopted to normalize the target name limiting the species to “*Homo sapiens*”. Interaction targets between active ingredient and retinal diseases were obtained through Bioinformatics platform (http://www.bioinformatics.com.cn/) and were defined as potential targets. The potential targets were then compared with five single TCMS separately. A Venn diagram chart was constructed to observe the intersection between the active ingredient and disease targets visually.

The protein-protein interaction (PPI) network was derived from the STRING database (https://string-db.org/). Potential targets were inputted into the STRING database with a confidence score of < 0.9 and species limited to “*Homo sapiens*”. Cytoscape (version 3.7.1) was applied to construct and visualize a PPI network by importing the PPI data and removing the non-connection genes. The network analyzer function was used for topological analysis, and key targets of the novel TCM prescription in the treatment of retinal diseases were acquired according to the twice the median degree. Furthermore, the top ten genes were chosen as core targets according to values of the degree.

Furthermore, gene ontology (GO) function enrichment, which includes biological processes (BPs), cellular components (CCs) and molecular functions (MFs), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were adopted to find out the functional category and signal pathways of the key targets. Enrichment analysis of GO and KEGG was performed by the DAVID database (https://david.ncifcrf.gov/) and a false discovery rate (FDR) of less than 0.05 was regarded as the rule in screen terms for retrieval results in GO function enrichment and KEGG pathway enrichment. The correlations with FDR < 0.05 were determined as significant correlations [24]. The top twenty biological functions identified with GO analysis and the top twenty pathways revealed by KEGG analysis were put in the Bioinformatics platform for mapping. The top ten pathways with the highest corrected FDR were considered as core pathways.

All potential active ingredients of the novel TCM prescription and the key targets were imported in analyzing tool of Cytoscape 3.7.1 software to create the network of potential active ingredients-key targets. Potential active ingredients superior to twice the median degree value were considered to be key active ingredients. Among these key active ingredients, ten active ingredients with a highest degree, which this study further analyzed as core active ingredients of the novel TCM prescription against retinal diseases. Subsequently, a network map of the key active ingredients-key targets pathways was constructed and the possible interactions between the targets of disease and the active ingredients of the novel TCM prescription were clarified. Finally, to explore the therapeutic mechanisms of novel TCM prescription in the therapy of different retinal diseases and mine information of biological value, the network of core TCMS-core active ingredients-core targets-core pathways was constructed.

**Molecular docking of core targets and core active ingredients of the novel TCM prescription against retinal diseases.** To assess the credibility of the association between core targets and core active ingredients of the novel TCM prescription for the treatment of retinal diseases, molecular docking was performed. The 3D structures of the core protein receptors and the core active components were retrieved from the RCSB PDB database (https://www.rcsb.org) and PubChem database (https://pubchem.ncbi.nlm.nih.gov/) respectively. The chosen condition of core target protein receptors for species and resolutions were “*Homo sapiens*” and 1.5–3.0 Å, respectively. Moreover, the 3D structures of the core active ingredients were obtained from the as ligands. AutoDock Vina 1.5.7 was then used to routinely process protein receptors and molecule ligands and obtain docking active sites for molecular docking to calculate binding energy. The PyMOL software (version 2.5.0) were applied to visualize results.

The binding energy threshold condition of ≤ −5.0 kcal/mol was deemed to the more favorable docking pose.

**Molecular dynamics simulation.** The software of GROMACS 2021 was adopted to accomplish MD simulation through analyzing the binding affinities of core targets and core active ingredients identified by molecular docking. Proteins were analyzed by the AMBER14SB force-field parameter while the active ingredients were analyzed by general AMBER force field. The SPC/E water model was selected, and periodic boundary conditions were set. Then, a water box was established to neutralize the charge by adding hydrogen atoms and antagonistic ions. A 50,000 steps of the steepest descent method was invested to get the maximization of energy in complex system, after that, system temperature was steadily increased from 0 K to 300 K at a fixed volume and a constant heating rate. A 100 ps NVT (isothermal and isovolume) system simulation was performed to uniformly distribute the solvent molecules in the solvent tank at a system maintenance temperature of 298.15 K. Finally, the system was subjected to MD simulation for 100 ns under the NPT ensemble with a time step of 2 fs. The root mean square deviation (RMSD), root mean square fluctuation (RMSF) and radius of gyration (Rg) of the MD simulation results were analyzed and visualized using the GROMACS embedded program and Visual Molecular Dynamics. MD simulation results such as RMSD, RMSF and Rg value lay the foundation of detecting stability of the complex systems of compounds and proteins.

**Results**

**Patent analysis**

Submit a manuscript: https://www.tmrjournals.com/tmr
Temporal and spatial distribution of TCM prescription patents containing *Chrysanthemi Flos* against retinal diseases. Based on the inclusion and exclusion criteria mentioned above, a total of 227 TCM prescription patents in the treatment of retinal diseases were obtained. Additionally, the number of patent applications for TCM prescriptions containing *Chrysanthemi Flos* against retinal diseases increased year by year (Supplementary Figure S1). From 1980 to 1992, the number of patent applications for TCM prescriptions containing *Chrysanthemi Flos* for retinal diseases increased gradually, with only a few pioneers working on retinal diseases. It indicated that the field of TCM treatment for retinal diseases had been at a technical germination period before 1992. From 1993 to 2004, the number of applications fluctuated at approximately five each year were regarded as the initial stage of retinal diseases research. Since 2004, the number of applications rapidly increased, exceeding 25 in 2014, reaching a peak.

The distribution of TCM prescription patents containing *Chrysanthemi Flos* for retinal diseases was geographically divergent in the provinces and municipalities of China (Supplementary Figure S2). Shandong province has the largest number of patents, followed by Beijing, Jiangsu province, Anhui province and Shannxi province, together accounting for 57%. Eight foreign patents were also found, accounting for 3.5% and involving the United States, Japan, Canada and the Netherlands, showing that TCM prescriptions containing *Chrysanthemi Flos* against retinal diseases have attracted worldwide interest.

Types of applicants for TCM prescription patents containing *Chrysanthemi Flos* against retinal diseases. The types of applicants for TCM prescription patents containing *Chrysanthemi Flos* against retinal diseases were statistically analyzed (Supplementary Figure S3), with individual applicants accounting for 61%, followed by enterprises, accounting for 27%. The number of applications for TCM prescription patents containing *Chrysanthemi Flos* against retinal diseases filed by research institutions, universities and medical establishments in this field was relatively small, at less than 10%. Thus, the study of TCM prescription patents for the treatment of retinal diseases belongs to the basic technical field and is conducted mostly by individual researchers.

Distribution of dosage forms for TCM prescription patents containing *Chrysanthemi Flos* against retinal diseases. Dosage forms for TCM prescription patents containing *Chrysanthemi Flos* against retinal diseases included topical and oral administration. Two types of topical drugs for the eyes were found: one used to treat retinal diseases of the anterior segment, such as conjunctivitis and GLA, mostly as eye drops; the other involved specific injections, vitreous injections and other methods for posterior segment diseases such as retinopathy and diabetic macular edema [25, 26]. Oral routes of administration for the TCM prescription patents containing *Chrysanthemi Flos* for the treatment of retinal diseases were primarily as capsules, decoctions, tablets, pills and granules, accounting for 88% of the total patents (Supplementary Figure S4). Owing to the unique physiological structure of the eye, retinal diseases have particular requirements for administration compared to other diseases, requiring a high degree of innovation in external administration. This was indeed observed in the patents, with eye drops and eye patches accounting for approximately 7% of the total patents, and eye gels, ophthalmic ointments, ophthalmic powders, membranes and injections as supplements accounting for approximately 5% of the total patents. Direct drug delivery to the eye faces great challenges and opportunities owing to its unique physiological structure [27].

Classifications of retinal diseases of TCM prescriptions containing *Chrysanthemi Flos* collected from patent databases. Herein, the patents were analyzed to display domestic and foreign research and patent layout. Patents were found for the treatment of various retinal diseases, including GLA, DR, AMD, retinal vein occlusion, central serous chorioretinopathy, retinitis pigmentosa, retinal artery occlusion and other retinal diseases (macular edema, retinal detachment, macular hole, etc.). The top three types of retinal diseases mentioned in the related patents were GLA, DR, and AMD (Figure 2).

World Health Organization reports that at least 2.2 billion people have the vision impairment affects globally [25]. GLA, DR and AMD are the leading causes of severe visual impairment and vision loss. According to the patents found, TCM prescriptions containing *Chrysanthemi Flos* have significant effects on the treatment of various retinal diseases. In our opinion, TCM prescriptions containing *Chrysanthemi Flos* achieved the effect of same treatment for different diseases through complicated regulation of multi-components and multi-targets. Therefore, below, we screened a novel TCM prescription containing *Chrysanthemi Flos* for the treatment of retinal diseases according to the patent databases and explore its potential molecular mechanisms focusing on the treatment of GLA, DR and AMD.

**Analysis of the medication rule of TCM prescriptions containing *Chrysanthemi Flos* collected from patent databases**

**Analysis of frequency and efficacy categories of TCMs in 227 TCM prescriptions containing *Chrysanthemi Flos* collected from patent databases.** Among the 227 TCM prescription patents containing *Chrysanthemi Flos* for use against retinal diseases included in the analysis, there were 374 kinds of TCMs contained. The 374 TCMs were used 3,361 times, and 24 of them were used over 35 times each. The top 24 high-frequency TCMs compatible with *Chrysanthemi Flos* were *Lycii Fructus* (121 times), *Cassiae Semen* (100 times), *Angelicae Sinensis Radix* (81 times), *Rehmanniae Radix Praparata* (69 times), *Salviae Miltiorrhizae Radix et Rhizoma* (68 times) and so on, suggesting their significant efficacy in the therapy of retinal diseases (Supplementary Table S1).

TCMs can be classified into 23 categories in terms of the 10th edition of Traditional Chinese Medicine. The classification of all 374 TCMs in 227 TCM prescriptions collected from patent databases containing *Chrysanthemi Flos* (Figure 3) showed that all TCMs contained in this study could be separated out of 21 categories. The top four TCM categories for treating retinal diseases were heat-clearing medicinals (78), supplementing medicinals (68), exterior-releasing medicinals (29) and blood-inovigating and stasis-dissolving medicinals (28). Thus, the basic principles for the treatment of retinal diseases were to enhance the body's immunity, promote blood circulation, relieve fever and reduce inflammation.

**Analysis on four natures, five tastes and meridian tropisms of TCMs in 227 TCM prescriptions containing *Chrysanthemi Flos* collected from patent databases.** Nature, tastes and meridian tropisms not only are the capabilities of TCMs but also are the basic properties and characteristics highly summarized in herbal efficacy [19]. The nature, taste and meridian status of the TCMs were analysed statistically (Figure 4). Bitter TCMs were the most common (184 times, 31.29%), followed by sweet TCMs (182 times, 30.95%) and acid TCMs (108 times, 18.37%) (Figure 4B). Most of the 374 TCMs were attributed to liver, lung, spleen and stomach meridian tropisms (Figure 4C). Among them, the TCMs of liver (216 times) and lung (130 times) meridian tropisms were the most frequently mentioned.

**Analysis of association rules of TCMs containing *Chrysanthemi Flos* collected from patent databases.** The relationship between TCM and TCM in the 227 prescriptions was analyzed by the method of association rules. A total of 110 medication rules of common medicine were acquired with support degree greater than 0.10 and lift degree greater than 1. Rules were sorted in order of decline based on the support level and frequency of occurrence, and drug pairs with a support degree greater than 20% were extracted (Supplementary Table S2). The effective and strongly related medicine combinations used frequently in the treatment of retinal diseases were *Chrysanthemi Flos – Lycii Fructus* (supplementing medicinals), *Chrysanthemi Flos – Cassiae Semen* (heat-clearing medicinals), *Chrysanthemi Flos – Angelicae Pubescens Radix* (supplementing medicinals), *Chrysanthemi Flos – Rehmanniae Radix Praparata* (supplementing medicinals) and *Chrysanthemi Flos – Salviae Miltiorrhizae Radix et Rhizoma* (blood-inovigating and stasis-dissolving medicinals). *Chrysanthemi Flos* was often used in combination with medicines to enhance the body's immunity, relieve fever, reduce inflammation, promote blood circulation and decrease swelling, in agreement with the above statistical results of frequency.
Screening of a novel TCM prescription for the treatment of retinal diseases. The algorithm of apriori was used for the analysis of association rule analysis, and the association rule graph was produced using SPSS Modeler 18.0 software. Degree values were interpreted based on the size and shade of the node – the larger and darker a node, the greater the importance of the TCM. Combined with the degree, closeness centrality and betweenness centrality analyses, TCMs with the most obvious color and size in the graph were Chrysanthemi Flos, Cassiae Semen, Lycii Fructus, Angelicae Sinensis Radix and Salviae Miltiorrhizae Radix et Rhizoma (Figure 5). The parameters of five core TCMs in the novel TCM prescription are shown in Table 1. Interestingly, the five core TCMs were characterized as a novel TCM prescription for the treatment of retinal diseases.

Analysis of the molecular mechanism of the novel TCM prescription against retinal diseases

Prediction of targets and potential active ingredients of the novel TCM prescription against retinal diseases. The potential active ingredients of five core TCMs in the novel TCM prescription for the treatment of retinal diseases were retrieved from the TCMSP databases and literature. A total of 362 active ingredients were obtained, including Chrysanthemi Flos (n = 78), Cassiae Semen (n = 29), Lycii Fructus (n = 125), Angelicae Sinensis Radix (n = 42) and Salviae Miltiorrhizae Radix et Rhizoma (n = 88). The details are described in Supplementary Tables S3-S7. On the basis of the potential active constituents of the novel TCM prescription, there were 2,448 targets acquired from the Swiss Target Prediction database, SEA Search Server database and PharmMapper database after removing...
duplicates.

Prediction of retinal diseases targets and acquisition of intersection targets of the novel TCM prescription and retinal diseases. The TTD, DrugBank, OMIM and DisGeNET databases were applied to forecast three retinal diseases related target genes. After removing duplicates, 812 target genes related to GLA were retrieved, among which 260 overlapping genes were identified as potential targets of GLA. The distribution of intersection targets between TCMs in the novel TCM prescription and GLA were obtained (Supplementary Figure S5A). With regards to DR, 585 targets were related and 271 potential targets of the novel TCM prescription and DR targets were obtained (Supplementary Figure S5B). A total of 651 AMD targets were obtained, and 207 overlapping genes were identified as potential targets of the novel TCM prescription against AMD (Supplementary Figure S5C). In addition, the intersecting targets for three retinal diseases and the novel TCM prescription were 47 common targets (Supplementary Figure SSD).

Construction of PPI network and screening of core targets of the novel TCM prescription against GLA. For understand the relationship among the target proteins visually, Cytoscape software was carried to build the PPI network among the 260 potential targets after eliminating disconnected nodes. At a confidence level of 0.9, the PPI network had 188 nodes and 661 edges were obtained. According to the degree value, 50 targets with a degree value greater than 9 (twice the median) were identified as key targets (Figure 6A). The PPI network contained 50 nodes and 294 edges, representing 294 interactions among the 50 targets. Furthermore, the top ten target proteins with the highest degree were considered core targets of the novel TCM prescription against GLA. These core targets were STAT3, EP300, TP53, RELA, AKT1, VFGEA, MAFK3, RHOA, PIK3CA and PIK3R1. The parameters of core targets in the novel TCM prescription for treating GLA are shown in Supplementary Table S8.

Construction of PPI network and screening of core targets of the novel TCM prescription against DR. Using the same way to construct the PPI network and filtrate DR targets. With a confidence of 0.9, 200 target proteins and 870 interaction relationships were obtained. After filtering with the network topological parameter degree, 55 key targets with a higher degree (degree ≥ 12) were identified. A PPI network with 55 nodes and 411 edges was built (Figure 6B). Additionally, the top ten targets with the highest degree were identified as core DR targets, including PIK3CA, RELA, AKT1, MAPK3, STAT3, CTNNB1, MAPK1, EP300, VFGEA and EGFR. The parameters of the core targets in the novel TCM prescription for the treatment of DR are listed in Supplementary Table S9.

Construction of PPI network and screening of core targets of the novel TCM prescription against AMD. The PPI network construction was completed in the same manner as for the described above. According to the confidence level, 155 targets and 443 interaction relationships were obtained. After screening with the thresholds of degree ≥ 8 (twice the median), 38 targets were taken as key targets of the novel TCM prescription for treating AMD, and a PPI network with 38 nodes and 185 edges was plotted (Figure 6C). Moreover, STAT3, PIK3CA, TP53, MAPK3, VFGEA, MAPK1, CTNNB1, IL10, IL6 and TGFβ1 were the core targets of the novel TCM prescription against AMD with the highest degree. Parameters of the core targets in the novel TCM prescription for treating AMD are summarized in Supplementary Table S10.

### Table 1 Topological information of five core TCMs in the novel TCM prescription for the treatment of retinal diseases

<table>
<thead>
<tr>
<th>Herb name</th>
<th>Degree value</th>
<th>Closeness centrality</th>
<th>Betweenness centrality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrysanthemi Flos (CHF)</td>
<td>95</td>
<td>1</td>
<td>0.484716</td>
</tr>
<tr>
<td>Cassiae Semen (CAS)</td>
<td>70</td>
<td>0.791667</td>
<td>0.110242</td>
</tr>
<tr>
<td>Lycii Fructus (LYF)</td>
<td>60</td>
<td>0.730769</td>
<td>0.052295</td>
</tr>
<tr>
<td>Angelicae Sinensis Radix (ANSR)</td>
<td>54</td>
<td>0.695829</td>
<td>0.025275</td>
</tr>
<tr>
<td>Salviae Miltiorrhiza Radix et Rhizoma (SMR)</td>
<td>49</td>
<td>0.673759</td>
<td>0.025275</td>
</tr>
</tbody>
</table>

![Figure 5](https://www.tmrjournals.com/tmr) Screen of a novel TCM prescription for the treatment of retinal diseases based on association rule analysis. Each node represents a TCM in the network. A bigger node means more important TCMs. Submit a manuscript: https://www.tmrjournals.com/tmr
**GO function and KEGG pathway analysis of the key targets of the novel TCM prescription against GLA.** For immersing in understanding the primary biological work of the novel TCM prescription in the treatment of GLA, the enrichment analysis of GO and KEGG pathway for the 50 key targets was conducted. Based on the retaining results (FDR < 0.05), 260 BPs, 18 CCs, 61 MFs and 154 KEGG pathways were obtained. For each GO enrichment, the first 10 items were visualized to generate a histogram. For the KEGG pathway, the first 20 items were visualized and analyzed to make a bubble chart. The vertical coordinate of the bubble diagram indicates the pathway name, and the horizontal coordinate indicates the gene ratio. The gene number was represented with the size of the bubble, and the shade of the bubble represents the FDR value. The darker the bubble, the greater the correct FDR value and the greater the enrichment significance, and the larger the bubble, the greater the number of genes. The key targets in the BP were mainly response to regulation of apoptotic process, response to regulation of gene expression and response to hypoxia. The CC of key targets were predominantly the nucleus, cytoplasm and transcription factor complex. The key targets for MF were mainly enzyme binding, transcription coactivator binding and transcription factor binding (Figure 7A). Through enrichment analysis of KEGG pathway, the key targets were mainly concentrated on the AGE-RAGE signaling pathway in diabetic complications, PI3K-Akt signaling pathway, HIF-1 signaling pathway and EGFR tyrosine kinase inhibitor resistance (Figure 7B).

**GO function and KEGG pathway analysis of the key targets of the novel TCM prescription against DR.** By conducting KEGG and GO analysis on 55 key targets of DR, a total of 291 BPs, 36 CCs, 58 MFs and 162 KEGG pathways were obtained (FDR < 0.05). Results of enrichment analysis showed that the BP mainly involved response to positive the regulation of ERK1 and ERK2 cascade, positive response to the regulation of gene expression, positive response to the regulation of cell migration and signal transduction. The CC were relevant to the extracellular region, cytoplasm and glutamatergic synapse. MF were mainly enriched in enzyme binding, identical protein binding, protein binding and insulin receptor substrate binding (Figure 8A). Analysis of KEGG pathway enrichment revealed that the key targets were mainly enriched in AGE-RAGE signaling pathway in diabetic complications, MAPK signaling pathway, HIF-1 signaling pathway and EGFR tyrosine kinase inhibitor resistance (Figure 8B).

**GO function and KEGG pathway analysis of the key targets of the novel TCM prescription against AMD.** Analysis of GO and KEGG enrichment were performed for the 38 key targets of AMD. According to the retaining results (FDR < 0.05), a total of 208 BPs, 16 CCs, 34 MFs and 134 KEGG pathways were obtained. The BP mainly enriched in response to regulation of gene expression, positive regulation of phosphatidylinositol 3-kinase signaling, positive regulation of cell migration, positive regulation of blood vessel endothelial cell migration, positive regulation of protein phosphorylation and positive regulation of apoptotic process. CC mainly involved extracellular region, macromolecular complex and blood microparticle. MF were mainly enriched in identical protein binding, growth factor activity, enzyme binding and cytokine activity (Figure 9A). Enrichment analysis of KEGG pathway indicated that the key targets were mostly involved in EGFR tyrosine kinase inhibitor resistance, the AGE-RAGE signaling pathway in diabetic complications, HIF-1 signaling pathway and PI3K-Akt signaling pathway (Figure 9B).

**Screening of key active ingredients and network construction of the key active ingredients-key targets-key pathways of the novel TCM prescription against GLA.** The 362 potential active ingredients of the novel TCM prescription and 50 key targets of GLA were imported into Cytoscape to establish the network of potential active ingredients-key targets, and the non-connection potential ingredients were removed. The final network consisted of 362 nodes and 1,940 edges (Supplementary Figure S6A). As suggested by the network topological analysis, 48 active components with degree ≥ 10 (twice the median) were identified as key active ingredients of the novel TCM prescription in the treatment of GLA. An efficacy network map of the TCMs-key active ingredients-key targets-key pathways-GLA was then constructed (Supplementary Figure S6B). Notably, the top ten active ingredients, namely (ferulic acid), A2 (caffeic acid), JH70 (hesperetin 7-O-glucoside), JM25 (chrysoerubtin), GQ124 (ethyl trans-ferulate), DS59 (isofurulic acid), GQ67 (N-trans-caffeoyltyramine), DS1 (2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofuranacarboxaldehyde), JH38 (eupatilin) and DG11 (4-hydroxychinamic acid), may play a critical role in the novel TCM prescription for the treatment of GLA given that they had the highest degree value. These ingredients included flavonoids, phenolic acids, anthraquinones, alkaloids and lignans.

**Screening of key active ingredients and network construction of the TCMs-key active ingredients-key targets-key pathways of the novel TCM prescription against DR.** The network of potential active ingredients-key targets for the treatment of DR was constructed (Supplementary Figure S7A). According to a degree of ≥ 12, 32 active ingredients were further screened and identified as key active ingredients of the novel TCM prescription for the treatment of DR. The network of TCMs-key active ingredients-key targets-key pathways-DR was then constructed (Supplementary Figure S7B). The top ten core active ingredients were A1 (ferulic acid), A2 (caffeic acid), B5 (isorhamnetin), JH8 (chrysoerol), JH21 (butyl octyl phthalate), JH38 (eupatilin), GQ124 (ethyl trans-ferulate), DG8 (coniferyl ferulate), DS59 (isofurulic acid) and DS1 (2-[4-hydroxy-3-methoxyphenyl]-5-[3-hydroxypropyl]-7-methoxy-3-benzofuranacarboxaldehyde), which included flavonoids, esters, phenolic acids and lignans.

**Screening of key active ingredients and network construction of the TCMs-key active ingredients-key targets-key pathways of the novel TCM prescription against AMD.** A network of potential active

---

**Figure 6 PPI network of the novel TCM prescription in the treatment of three types of retinal diseases including (A) GLA, (B) DR and (C) AMD. Each node represents a target in the PPI network, and each edge indicates the interaction between adjacent nodes. The greater the number of adjacent nodes, the greater the probability of becoming a core target.**

Submit a manuscript: https://www.tmrjournals.com/tmr
The top ten core active ingredients were A1 (ferulic acid), A2 (caffeic acid), B4 (luteolin), B5 (isorhamnetin), JH6 (acacetin), JH38 (eupatilin), JM10 (obtusifolin), JM25 (chrysooobtusin), GQ1 (acetyl-L-tryptophan) and DS59 (isofurulic acid), which included flavonoids, phenolic acids, anthraquinones and amino acids.
Network analysis of core TCMs-core active ingredients-core targets-core pathways of the novel TCM prescription against retinal diseases. To further study the correlation of the novel TCM prescription in the treatment of different retinal diseases, the network of core TCMs-core active ingredients-core targets-core pathway was constructed for the treatment of three retinal diseases by the novel TCM prescription. This network involved a total of eighteen core active ingredients, sixteen core targets and fifteen core pathways (Figure 10). Details of the core active ingredients and core targets are shown in Tables 2, 3. The 2D structures of eighteen core active ingredients are shown in Figure 11. Importantly, the novel TCM prescription played its role in treating retinal diseases through various routes, involving multiple gene targets and signaling pathways. Several pathways were crosslinked through same targets, suggesting a synergistic effect of the core active ingredients of the novel TCM prescription in the treatment of retinal diseases.

Molecular docking results of core active ingredients and core targets of the novel TCM prescription against retinal diseases. To better understand the interactions between the core active ingredients and core targets, a molecular docking analysis was performed between eighteen core active ingredients and sixteen core targets. The lower the binding free energy between the ligand and the receptor, the more stable the conformation, indicates a stronger affinity between them [28]. In general, when the binding free energy is lower than 0, the ligand and receptor bind spontaneously. Herein, the binding free energy was below ~5 kcal/mol, indicating good binding activity and a stable binding conformation [29]. The docking scores of core active ingredients and core targets of the novel TCM prescription obtained by molecular docking are shown in Figure 12A. Almost all of core active ingredients had strong binding activities with core targets, and the binding conformation was stable. Molecular docking validated the reliability of results derived from network pharmacology. The representative molecular docking results were visualized by PyMOL (Figure 12B), further suggesting that the novel TCM prescription may treat retinal diseases through these core active ingredients and targets.

Molecular dynamics simulation. MD simulation can predict the stability of protein-ligand complexes. In this study, based on the molecular docking results, the core active ingredients in five core TCMs binding with the core targets in a lowest energy were subjected to perform MD simulation analysis for 100 ns to evaluate the motion, trajectory, structural features, binding potential, and conformational changes in molecular level. RMSD is a good measure of the conformational stability of the protein-ligand complex and is a measure of the extent of deviation in the position of atoms from the starting position. A lower deviation indicates a better conformational stability. The RMSD curves of the protein and the ligand during the 100 ns MD simulations of docked complexes are shown in Figure 13A, Supplementary Figures S9–S13. The Rg curve represents the degree of compactness of the overall structure of the protein, and the Rg curves of the MD process of docked complexes are shown in Figure 13B. The RMSF of the amino acid residues was analyzed to determine the flexibility of the residues. RMSF curves of the MD process of the docked complexes are shown in Figure 14.

For the RMSD of the MD simulations (brown broken line shown in Figure 13A), it is not good that the overall performance of the PIK3R1 with N-trans-cafeoyltyramine complex system in the five MD systems of the compound-target complexes. Although the RMSD trajectory of the PIK3R1 with N-trans-cafeoyltyramine complex fluctuated, it eventually became stable. However, the PIK3R1-obtusifolin complex displayed a large fluctuation of up to 25 ns and was slowly stabilized around 1 nm at the end of the simulation (blue broken line shown in Figure 13A). For the three complex systems (purple, red and green broken lines shown in Figure 13) formed by PIK3CA-hesperetin-7-O-glucoside, EGFR-luteolin and PIK3CA-coniferyl ferulate, experimental Rg results in the EGFR-luteolin system were lower than those of the other two systems, but all the three systems performed well. They could exist steadily with itself structure and do not influence the construction of other proteins. In terms of RMSD, Rg and RMSF, the MD system (red broken line shown in Figures 13, 14) formed by EGFR-luteolin showed a relatively good state. It had the most stable RMSD and the smallest Rg. Therefore, PIK3CA and EGFR were possible novel therapeutic targets for retinal diseases. Our study confirmed that the core active ingredients could target the protein well. Results of the present study illustrate that the novel TCM prescription may facilitate to improve or treat retinal diseases to a certain extent by acting on the core target.
Retinal diseases, a leading cause of blindness, are becoming a global crisis with the number of people with retinal diseases expected to more than double by 2050 [30]. Thus, there is an urgent need to address the prevention and treatment of retinal diseases. According to the TCM theory, the impairment of liver and kidney function is an intrinsic factor in the occurrence of retinal diseases. For example, the main pathogenesis of GLA includes decreased liver and kidney function, depression and fatigue [31]. The pathogenesis of DR is mainly due to sluggish and unsmooth blood circulation caused by endocrine disorders [32]. TCMs with heat-clearing (reducing the body's fever and inflammatory response) and supplementing (enhancing the body's immunity) activities have significant therapeutic effects on retinal diseases [13]. For example, Andrographis Herba, a representative heat-clearing medicine, ameliorates DR by inhibiting retinal angiogenesis and inflammation [33]. Astragali Radix, a TCM with supplementing activities, can alleviate dysfunction of the retinal ganglion (RGG) cells in the treatment of DR [34]. The Chinese medicine classifies ocular diseases as a hepatic disease. The liver meridian plays a significant role in linking the eye with the liver as well as running of Qi and blood (Qi-blood is the material basis for the physiological activities of viscera, meridian and other organs in human body. The formation and operation of Qi-blood depends on the normal physiological function of viscera.). In this system, the liver is the main reservoir of blood and the eyes need nutrient supply through blood flow [35]. In addition, the Five Wheel Theory, an ancient doctrine of ophthalmology in TCM, divides the Baijing of the eye including the bulbarch conjunctiva and the sclera, dominated by the lung (the gas wheel) [35]. The lung channel is an important meridian for the treatment of retinal diseases. TCMs screened in this study for the treatment of retinal diseases mainly belong to the liver and lung meridian tropisms, which was consistent with the TCM theory. In the current study, a novel TCM prescription consisting of Chrysanthemi Flos, Cassiae Semen, Lycii Fructus, Angelicae Sinensis Radix and Salviae Miltiorrhiza Radix et Rhizoma was shown to have potential therapeutic effect on three types of retinal diseases, namely GLA, DR and AMD. A previous study demonstrated that Chrysanthemi Flos protects RPE cells from light damage and alleviates the progression of AMD [36]. Lycii Fructus has been shown to ameliorate retinal structural and functional changes due to diabetes [37]. Combined Chrysanthemi Flos and Lycii Fructus effectively prevented retinal damage caused by diabetes and played a favorable therapeutic effect on diabetic rats with retinopathy [38]. Cassiae Semen, as a potential drug for the treatment of DR, protected against high glucose-induced retinal endothelial cell injury [39]. Angelicae Sinensis Radix was known as the assistant drug for the improvement of eyesight and plays a significant role in protecting retinal function in patients with DR [31, 40]. Salviae Miltiorrhiza Radix et Rhizoma have been shown to have a protective effect on DR mice through the blood-ocular barrier [41]. These studies coincided with the findings herein that these five TCMs are of importance in the treatment of retinal diseases.

Table 2 Basic information of core active ingredients in the novel TCM prescription for the treatment of retinal diseases

<table>
<thead>
<tr>
<th>No.</th>
<th>Core active ingredients</th>
<th>PubChem ID</th>
<th>Primary diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (JH69, GQ2, DG1, DS10)</td>
<td>Ferulic acid</td>
<td>445858</td>
<td>GLA, DR, AMD</td>
</tr>
<tr>
<td>A2 (JH46, GQ11, DG27, DS39)</td>
<td>Caffeic acid</td>
<td>689043</td>
<td>GLA, DR, AMD</td>
</tr>
<tr>
<td>B4 (JH3, DS43)</td>
<td>Luteolin</td>
<td>5280445</td>
<td>AMD</td>
</tr>
<tr>
<td>B5 (JH32, GQ20)</td>
<td>Isohamnetin</td>
<td>5281654</td>
<td>DR, AMD</td>
</tr>
<tr>
<td>JH6</td>
<td>Acacetin</td>
<td>5280442</td>
<td>AMD</td>
</tr>
<tr>
<td>JH8</td>
<td>Chrysoeriol</td>
<td>5280666</td>
<td>DR</td>
</tr>
<tr>
<td>JH12</td>
<td>Butyl octyl phthalate</td>
<td>66540</td>
<td>DR</td>
</tr>
<tr>
<td>JH38</td>
<td>Eupatin</td>
<td>5273755</td>
<td>GLA, DR, AMD</td>
</tr>
<tr>
<td>JH70</td>
<td>Hesperetin-7-O-glucoside</td>
<td>147394</td>
<td>GLA</td>
</tr>
<tr>
<td>JM10</td>
<td>Obutisolin</td>
<td>3083575</td>
<td>AMD</td>
</tr>
<tr>
<td>JM25</td>
<td>Chrysoobutin</td>
<td>155381</td>
<td>GLA, AMD</td>
</tr>
<tr>
<td>GQ1</td>
<td>Acetyl-L-tryptophan</td>
<td>700653</td>
<td>AMD</td>
</tr>
<tr>
<td>GQ67</td>
<td>N-trans-cafeoyltyramine</td>
<td>9994897</td>
<td>GLA</td>
</tr>
<tr>
<td>GQ124</td>
<td>Ethyl trans-furulate</td>
<td>736681</td>
<td>GLA, DR</td>
</tr>
<tr>
<td>DG8</td>
<td>Coniferyl furulate</td>
<td>6441913</td>
<td>DR</td>
</tr>
<tr>
<td>DG11</td>
<td>4-Hydroxycinnamic acid</td>
<td>637542</td>
<td>GLA</td>
</tr>
<tr>
<td>DS1</td>
<td>2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)</td>
<td>6709746</td>
<td>GLA, DR</td>
</tr>
<tr>
<td>DS59</td>
<td>Isoferulic acid</td>
<td>736186</td>
<td>GLA, DR, AMD</td>
</tr>
</tbody>
</table>

GLA, glaucoma; DR, diabetic retinopathy; AMD, age-related macular degeneration.

Table 3 Basic information of core targets and the numbers of related core pathways in the novel TCM prescription for the treatment of retinal diseases

<table>
<thead>
<tr>
<th>Core targets</th>
<th>Uniprot ID</th>
<th>Relevant core pathway numbers</th>
<th>Primary diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK3CA</td>
<td>P42336</td>
<td>13</td>
<td>GLA, DR, AMD</td>
</tr>
<tr>
<td>EP300</td>
<td>Q09472</td>
<td>5</td>
<td>GLA, DR</td>
</tr>
<tr>
<td>AKT1</td>
<td>P31749</td>
<td>14</td>
<td>GLA, DR</td>
</tr>
<tr>
<td>RHOA</td>
<td>P61586</td>
<td>4</td>
<td>GLA</td>
</tr>
<tr>
<td>RELA</td>
<td>Q04206</td>
<td>12</td>
<td>GLA, DR</td>
</tr>
<tr>
<td>MAPK1</td>
<td>P28482</td>
<td>14</td>
<td>DR AMD</td>
</tr>
<tr>
<td>VEGFA</td>
<td>P15692</td>
<td>10</td>
<td>GLA, DR, AMD</td>
</tr>
<tr>
<td>MAPK3</td>
<td>Q16644</td>
<td>15</td>
<td>GLA, DR, AMD</td>
</tr>
<tr>
<td>STAT3</td>
<td>P40763</td>
<td>10</td>
<td>GLA, DR, AMD</td>
</tr>
<tr>
<td>TP53</td>
<td>P04637</td>
<td>9</td>
<td>GLA, AMD</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>P35222</td>
<td>5</td>
<td>DR, AMD</td>
</tr>
<tr>
<td>EGFR</td>
<td>P00535</td>
<td>7</td>
<td>DR</td>
</tr>
<tr>
<td>IL6</td>
<td>P05231</td>
<td>11</td>
<td>AMD</td>
</tr>
<tr>
<td>IL10</td>
<td>P22301</td>
<td>1</td>
<td>AMD</td>
</tr>
<tr>
<td>PIK3R1</td>
<td>P27986</td>
<td>12</td>
<td>GLA</td>
</tr>
<tr>
<td>TGFB1</td>
<td>P01137</td>
<td>7</td>
<td>AMD</td>
</tr>
</tbody>
</table>

GLA, glaucoma; DR, diabetic retinopathy; AMD, age-related macular degeneration.
Figure 10 Network construction of the TCMs-core active ingredients-core targets-core pathways of the novel TCM prescription against three retinal diseases. The yellow hexagons represent TCMs of novel TCM prescription, the purple parallelograms represent the unique core active ingredients of different TCMs, the red parallelograms represent the common core active ingredients of different TCMs, the blue diamonds represent the core targets, the orange V shapes represent the core pathways and the green triangles represent the retinal diseases. GLA, glaucoma; DR, diabetic retinopathy; AMD, age-related macular degeneration.

Figure 11 Chemical structures of eighteen core active ingredients of the novel TCM prescription against retinal diseases

Figure 12 Molecular docking results of core active ingredients and core targets of the novel TCM prescription for the treatment of retinal diseases. (A) Heatmap of the docking scores of core active ingredients and core targets of the novel TCM prescription. (B) Representative 3D molecular docking structures of core active ingredients and core targets of the novel TCM prescription. The yellow lines represent hydrogen bond interaction force.
Figure 13 MD simulation results of five core active ingredients and core targets of the novel TCM prescription for the treatment of retinal diseases. (A) The RMSD curves of the molecular dynamics process of docked complexes. (B) The Rg curves of the molecular dynamics process of docked complexes. RMSD, root mean square deviation; Rg, radius of gyration.

Figure 14 The RMSF curves of the molecular dynamics process of docked complexes. RMSF, root mean square fluctuation.

Herein, network pharmacology was further used to analyze the possible molecular mechanisms of the novel TCM prescription in the treatment of retinal diseases. Our study indicated eighteen core active ingredients of the novel TCM prescription including six flavonoids, six phenolic acids, two anthraquinones, one ester, one amino acid, one alkaloid and one lignan, were involved in the treatment of retinal diseases, likely based on the direct and indirect regulation of sixteen core targets and fifteen core pathways. Inflammatory events and oxidative stress play vital roles in the pathogenesis of retinal diseases, yet the novel TCM prescription screened herein modulated the inflammatory response and oxidative stress response and could alleviate the occurrence and development of retinal diseases. Ferulic
acids, the major active compound in Angelicae Sinensis Radix, has anti-inflammatory effects and can attenuate microglia-mediated neuroinflammation in retinal degeneration [42]. AKT regulates multiple signaling pathways involved in cytotoxic drug-induced inflammation, apoptosis and autophagy [43]. Luteolin, the main active compound in Chrysanthemi Flos and Salviae Miltiorrhizae Radix et Rhizoma, induces AKT phosphorylation, thus suppressing MAPK inflammatory pathways [44]. The AGE-RAGE signaling pathway in diabetic complications is associated with oxidative stress and inflammation of retinal diseases. The accumulation of advanced glycation end products in vascular tissues leads to continuous oxidative stress, which could also bind and activate the receptor for advanced glycation end products, NADPH oxidase and NF-κB, simultaneously initiating a vicious cycle of oxidative stress and inflammation. Quercetin, the major ingredient in Chrysanthemi Flos and Lycii Fructus, might activate the AGE-RAGE signaling pathway by regulating the expression of JUN, MAPK1 and STAT3, exerting antioxidant stress activity and thus playing a major role in the treatment of DR [45]. EGFR might be an important target for the prevention of oxidative injury in RPE cells. The results of molecular docking showed that all the eighteen active ingredients in the novel prescription could effectively bind to the EGFR, AKT, MAPK and STAT3 targets, suggesting that above targets might be crucial targets of the novel prescription against retinal diseases.

Moreover, the novel TCM prescription might treat retinal diseases by suppressing retinal neovascularization. Naringenin, the main active ingredient in Chrysanthemi Flos, has anti-inflammatory effects, which could inhibit diabetic ocular neovascularization [46]. VEGFA is not only the most significant causative agent of vascular leakage, but is also a key angiogenic agent with a significant impact on the incidence of retinal diseases [47, 48]. HIF has antagonists that have a significant inhibitory effect on ocular neovascularization [49]. Obetinolpin, the core active ingredient in Cassiae Semen, has been shown to suppress retinal angiogenesis by downregulating the expression of HIF-1α and VEGF [50]. Expression of PIK3CA was related to the proliferation, migration and angiogenesis of microvascular endothelial cells in DR [51]. Furthermore, Cassiae Semen ethanol extract has been shown to reverse the up-regulation of EGFR and the down-regulation of PIK3CA [52]. Taken together, the novel TCM prescription could treat retinal diseases by inhibiting retinal neovascularization through the regulation of HIF-1α, VEGFA, EGFR and PIK3CA expression.

The apoptosis of RPE cells and RGC cells, as the pivotal pathological processes in retinal diseases, inevitably accelerates the development of retinal diseases. Quercetin has been shown to suppress apoptosis of RPE cell by activation of PI3K-AKT signaling pathway [53]. Isorhamnetin, the active ingredient of Chrysanthemi Flos and Lycii Fructus, eliminates reactive oxygen species production and inhibits oxidative stress-induced apoptosis to protect RPE cells through the PI3K-AKT signaling pathway [54]. Studies have reported that the apoptosis of RGC cells were reduced by quercetin through suppressing expression of TP53 [55]. In addition, retinal dysfunction, retinal structural impairment and retinal vascular abnormalities in DR can be alleviated by the inhibition of EGF [56]. Molecular docking results showed that eighteen active ingredients of the novel prescription could bind vigorously to the TP53 and EGFR targets, demonstrating that TP53 and EGFR were the most likely mediators of the novel prescription against retinal diseases. Noteworthy, PIK3CA and EGFR, which were considered the most important targets in the novel prescription against retinal diseases, bind to core active ingredients most stably, which was further validated by MD simulation. At present, the primary treatment drugs for retinal diseases are directed against VEGF including Macugen (pegaptanib sodium), Lucentis (ranibizumab) and Avastin (bevacizumab). Some side effects are associated with the use of these drugs, such as fibrovascular constriction and a persistent increase in intraocular pressure [57]. The novel TCM prescription screened herein for the treatment of retinal diseases modulated the sixteen core targets including STAT3, MAPK3, AKT1, PIK3CA, VEGFA, EGFR and TP53, which may involve fifteen core pathways. It embodies the advantages of TCM for treating retinal diseases with multiple targets and pathways. The combination of TCM and western medicine has been widely used in various disciplines, which is a major advantage of clinical medicine in China. However, the active ingredients and pharmacological effects of TCMs are very complex, and improper compatibility with western medicine may lead to physical discomfort of patients and, sometimes, serious medical accidents. The main drugs for the treatment of retinal diseases include ocular hypotensive agents, neuroprotectants and VEGF antagonists. The combination of the above western medicine and the novel TCM prescription may involve drug interaction to some extent, but further study on this topic is needed.

Our study preliminarily demonstrated that the novel TCM prescription has great potential in the treatment of retinal diseases. However, the dosage of the novel TCM prescription selected in this study and the ratio between different TCMs are still unclear. In the future, we will optimize the optimal ratio between different TCMs in the novel TCM prescription through orthogonal experiments. Animal and cell models will also be used to reveal the optimal dose at which the novel TCM prescription would work. On this basis, relevant clinical trials will be conducted to further prove the clinical efficacy of the novel TCM prescription in the treatment of retinal diseases and provide a scientific basis for its clinical application.

Conclusion

In this study, an integrated strategy of data mining, network pharmacology and molecular docking was adopted to screen a novel TCM prescription against retinal diseases from patents containing Chrysanthemi Flos and to explore its medication rule and molecular mechanism. Firstly, a total of 227 TCM prescriptions for the treatment of various retinal diseases were collected based on patent databases. The TCMs of these prescriptions were primarily composed of bitter and cold properties with heat-clearing and supplementing effects, which were mainly associated with the liver and lung meridian tropisms. Significantly, a novel TCM prescription composed of five TCMs was screened for the first time, with potential clinical value for the treatment of three types of retinal diseases, namely GLA, DR and AMD. Subsequently, network pharmacology was used to illustrate the molecular mechanism of the novel TCM prescription against retinal diseases. Eighteen compounds including six flavonoids, six phenolic acids, two anthraquinones, one ester, one amino acid, one alkaid and one lignan were regarded as core active ingredients of the novel TCM prescription. These core active ingredients were shown to suppress the inflammatory response, oxidative stress, retinal neovascularization, apoptosis of RGC cells and RPE cells, and improve retinal structural impairment by acting on the sixteen core targets, involving fifteen core signaling pathways. The molecular docking and MD simulation results indicated that the affinity between the majority of the core active ingredients and the core targets was good. To the best of our knowledge, this is the first study to screen a novel TCM prescription containing Chrysanthemi Flos against retinal diseases and to explore its molecular mechanism, and thus provides new ideas and references for the development of TCMs in the treatment of retinal diseases.

References


