RESEARCH ARTICLE

Discovery of quality markers and quality control mechanisms of Xiaosiwu Decoction based on network pharmacology and multi-omics

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Nephrotic syndrome is a clinical syndrome characterized by massive proteinuria, hypoalbuminemia, edema, and hyperlipidemia. Xiaosiwu Decoction is made by combining Xiaochaihu Decoction, Siwu Decoction, and Wuling Powder and is widely used in clinical treatment of kidney diseases. However, due to its complex chemical composition, the current single indicator quality control is difficult to reflect the overall efficacy. This study explored the quality markers of Xiaosiwu Decoction in the treatment of kidney disease based on network pharmacology and multi-omics and established a quality control method that reflected its overall efficacy. Network pharmacology was used to construct a "component-target-pathway" regulatory network. An adriamycin-induced nephropathy rat model was constructed to evaluate the pharmacological effects of Xiaosiwu Decoction on renal function, inflammatory responses, and oxidative stress injury. Chemical pattern recognition and multicomponent quantitative analysis were employed to identify potential quality markers. A total of 72 compounds were detected, and 28 compounds entered the blood circulation as prototype components. Network pharmacology yielded 112 potential targets. The core targets were involved in key signaling pathways related to cell survival, inflammatory regulation, and kinase mediated signal transduction. In the high-dose Xiaosiwu Decoction group, serum blood urea nitrogen decreased from 18.53 to 8.51 mmol/L and creatinine decreased from 98.74 to 48.62 μmol/L. Xiaosiwu Decoction also inhibited inflammatory cytokines and regulated oxidative stress indicators. Chemical pattern recognition indicated that several characteristic components were primarily responsible for batch differentiation with their prototype forms detected in blood samples. Baicalin, glycyrrhizic acid, ferulic acid, and pachymic acid were identified as core quality markers of Xiaosiwu Decoction, providing an experimental basis for its quality control.

Keywords: Xiaosiwu Decoction; quality marker; network pharmacology; metabolomics.

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Introduction

Xiaosiwu Decoction originates from the empirical combined formula, which is derived from the modification of three classical formulas including Xiaochaihu Decoction, Siwu Decoction, and Wuling Powder and possesses the effects of harmonizing Shaoyang, promoting blood circulation, and promoting diuresis [1, 2]. Clinical research showed that this prescription had a definite curative effect on nephrotic syndrome, diabetic nephropathy, lupus nephritis, and other

renal diseases [3, 4]. During treatment, Xiaosiwu Decoction can effectively reduce urinary protein levels, improve renal function indicators, and positively regulate hemorheological parameters [5, 6]. Nevertheless, the chemical composition of Xiaosiwu Decoction is highly complex, and current investigations regarding its quality control usually rely on single indicators, failing to provide a comprehensive evaluation of the overall quality of this multicomponent prescription [7, 8]. There is an urgent need for a quality marker evaluation method for Xiaosiwu Decoction.

Quality markers (Q-Markers) of traditional Chinese medicine (TCM) can reflect the effectiveness, specificity, transmissibility, traceability, and measurability of TCM, providing new insights for quality control [9, 10]. Wang *et al*. enhanced the quality control of Shancigu, a multi-origin TCM, through plant metabolomics, targeted isolation, structural identification, and bioactivity assays. The study screened five Q-markers and established a quality evaluation method based on single standard to determine multi-components, effectively distinguishing authentic products from counterfeits [11]. Zheng *et al*. predicted Q-markers of Coptis chinensis by combining systems pharmacology and plant phylogenetic relationships to screen active ingredients, identified alkaloids including berberine and coptisine and organic acids including quinic acid and malic acid as core Q-markers, and updated biosynthetic pathways [12]. Network pharmacology can help reveal the mechanisms of action of compound formulas and is commonly utilized to identify Q-markers [13, 14]. Chen *et al*. proposed an improved database assisted ultraperformance liquid chromatography (UPLC) quality analysis strategy. Through component identification of freeze-dried *Ginkgo biloba* extract powder combined with quantum chemical calculations, network pharmacology, and molecular simulation, the study screened seven core Q-markers [15]. Zhang *et al*. employed fingerprinting combined with similarity analysis to screen candidate components to clarify the potential Q-markers and pharmacodynamic targets of *Saposhnikovia divaricata*. Core targets were predicted through network pharmacology. The results showed that three chromone glycosides bounded well to targets such as mitogen-activated protein kinase (MAPK) [16]. Multi-omics technology is capable of identifying the pharmacodynamic material basis and network regulation of compound formulas through exogenous components and endogenous metabolites, playing a complementary role in network pharmacology [17, 18]. He *et al*. elucidated the mechanism of action of rhubarb in treating chronic kidney disease by ensuring quality consistency through fingerprinting, identifying key targets *via* a combination of network pharmacology and Mendelian randomization, and validating therapeutic efficacy through animal experiments. The results showed that rhubarb restored renal function by regulating the NF-κB signaling pathway [19]. Wang *et al*. explored the pharmacodynamics of Simiao Pill in rheumatoid arthritis using metabolomics and proteomics integrated for screening bioactive compounds and verifying through synovial cell experiments. *Phellodendrine* was found to have the potential to suppress inflammatory cytokines and serve as the main pharmacodynamic component of Simiao Pill [20]. Although previous studies have made progress in discovering quality markers for traditional Chinese medicine, they mostly focus on a single dimension. In addition, chemical analysis, network pharmacology, and metabolomics are often carried out independently, lacking a complete validation strategy spanning from chemical composition, blood absorption, and metabolic regulation to pharmacodynamic validation.

To address the issues of undefined quality markers and fragmented analytical strategies in Xiaosiwu Decoction research, this study adopted an integrated approach combining chemomics, serum metabolomics, network pharmacology, and *in vivo* pharmacodynamic validation for Q-marker discovery and quality control. The research established a nephropathy rat model and employed UPLC combined with quadrupole

time-of-flight tandem mass spectrometry (UPLC-Q-TOF-MS/MS) to identify the chemical constituents and absorbed metabolic components of Xiaosiwu Decoction. Meanwhile, animal experiments were conducted to verify its protective effects against inflammation, oxidative stress, and renal injury. Furthermore, candidate components were screened, thereby facilitating the discovery of Q-markers and the establishment of a quality control system for Xiaosiwu Decoction. This study aimed to obtain the core Q-markers of Xiaosiwu Decoction and explore its quality control mechanisms, providing a scientific basis for establishing quality standards of Xiaosiwu Decoction and offering a reference technical pathway for Q-marker research of TCM compound formulas.

Materials and methods

Preparation of Xiaosiwu Decoction freeze-dried powder

Xiaosiwu Decoction consisted of 16 TCMs including *Bupleurum chinense* DC. (12 g), *Scutellaria baicalensis* Georgi (9 g), *Codonopsis pilosula* (Franch.) Nannf. (9 g), *Pinellia ternata* (Thunb.) Breit. (9 g), *Glycyrrhiza uralensis* Fisch. (6 g), *Zingiber officinale* Rosc. (6 g), *Ziziphus jujuba* Mill. (6 g), *Polyporus umbellatus* (Pers.) Fries (9 g), *Alisma plantago-aquatica* L. (9 g), *Atractylodes macrocephala* Koidz. (9 g), *Poria cocos* (Schw.) Wolf (9 g), *Cinnamomum cassia* Presl (6 g), *Angelica sinensis* (Oliv.) Diels (9 g), *Rehmannia glutinosa* (Gaertn.) Libosch. (12 g), *Ligusticum chuanxiong* Hort. (6 g). All medicinal materials were purchased from Sichuan Qianfang Traditional Chinese Medicine Co., Ltd. (Chengdu, Sichuan, China) and were identified to comply with the requirements of the Chinese Pharmacopoeia (2020 Edition). Five reference standards including baicalin, glycyrrhizic acid, ferulic acid, pachymic acid, and saikosaponin A were obtained from the National Institutes for Food and Drug Control (NIFDC) (Beijing, China). The crude herbs were weighed and soaked in 10 volumes (v/w) of water for 30 min, boiled, and then simmered over low heat for 30 min followed by filtration. The residue was decocted with 8 volumes of water for an additional 20 min. The two filtrates were combined and concentrated under reduced pressure to a concentration of 1.0 g crude drug/mL using a DLAB RE100-S rotary evaporator (DLAB Scientific Co., Ltd., Beijing, China) and then lyophilized using a SCIENTZ-25T freeze dryer (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, Zhejiang, China) to obtain the lyophilized powder. Three batches of lyophilized powder were prepared following the same procedure for content determination and quality marker screening.

Animal nephropathy model construction

Specific pathogen-free (SPF) male Sprague-Dawley (SD) rats weighing 180 - 220 g were purchased from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, Hunan, China). All rats were housed in an SPF animal laboratory under controlled conditions of 22 ± 2℃, 50 ± 10% humidity, and a 12 h light/dark cycle with free access to food and water. Following one week of acclimatization, nephrotic syndrome was induced by a single tail vein injection of 2 mg/mL doxorubicin (brand name Adriamycin) at a dose of 6.5 mg/kg and administered within 10 s [21]. The animals were randomly divided into six groups with 10 animals per group including normal control group (Control), model group (Model), Xiaosiwu Decoction low-dose (3.5 g crude drug/kg) group (XSD-L), medium-dose (7.0 g crude drug/kg) group (XSD-M), high-dose (14.0 g crude drug/kg) group (XSD-H), and prednisone group (Pred) (6 mg/kg). All groups received intragastric administration at a volume of 10 mL/kg for 4 consecutive weeks. After the last administration, the rats were fasted for 12 h with free access to water and then anesthetized by intraperitoneal injection of 1% sodium pentobarbital at a dose of 40 mg/kg. Blood samples were collected from the abdominal aorta, allowed to stand for 30 min, and then centrifuged at 3,000 rpm for 15 min using a H1650-W high-speed centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Ltd., Changsha, Hunan, China) to separate the serum. The serum was aliquoted and stored at −80℃. All

procedures were approved by the Laboratory Animal Ethics Committee of Southern Medical University (Southern Medical University, Guangzhou, Guangdong, China).

Determination of biochemical indicators

Serum levels of blood urea nitrogen (BUN) and creatinine (Cr) were measured using a Hitachi 7180 automatic biochemical analyzer (Hitachi High-Tech Corporation, Tokyo, Japan). Serum levels of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-1 beta (IL-1β), superoxide dismutase (SOD), and malondialdehyde (MDA) were determined using enzyme-linked immunosorbent assay (ELISA) kits (Wuhan Fine Biotech Co., Ltd., Wuhan, Hubei, China) following the manufacturer's instructions.

Analysis of chemical components and metabolomics

20 mg of Xiaosiwu Decoction lyophilized powder was dissolved in 10 mL of 70% methanol and ultrasonicated for 30 min using a KQ-500DE ultrasonic cleaner (Kunshan Ultrasonic Instruments Co., Ltd., Kunshan, Jiangsu, China). The solution was filtered through a 0.22 μm microporous membrane. Blood samples were collected from XSD-M group at 0.5, 1, 2, and 4 h post-administration and pooled. 200 μL of plasma was mixed with 800 μL of acetonitrile and centrifuged at 12,000 rpm for 10 min at 4℃. The supernatant was evaporated to dryness under nitrogen gas and reconstituted in 100 μL of initial mobile phase for analysis. Blank control samples were prepared following the same procedure. UPLC-Q-TOF-MS/MS analysis was performed on a Waters ACQUITY UPLC system (Waters Corporation, Milford, MA, USA) coupled with an AB Sciex TripleTOF 6600+ mass spectrometer (SCIEX, Framingham, MA, USA). The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B). The gradient elution program was 0 – 2 min of 5% B, 2 – 20 min of 5 – 95% B, 20 – 25 min of 95% B, 25 – 25.1 min of 95 – 5% B, and 25.1 – 30 min of 5% B. The flow rate was 0.3 mL/min with the column temperature of 40℃ and the injection volume of 2 μL. Mass spectrometry was performed on the AB Sciex TripleTOF 6600+ system equipped with an electrospray ionization (ESI) source, operating in both positive and negative ion modes. The ion source temperature was set at 550℃, while the curtain gas, nebulizer gas (GS1), and auxiliary heating gas (GS2) were set at 30 psi, 50 psi, and 50 psi, respectively. The ion spray voltage was ±5,500 V, and the declustering potential was ±60 V. The TOF MS scan range was m/z 100 – 2,000, and the product ion scan range was m/z 50 – 1,500. The absorbed prototype components were identified by comparing the total ion chromatograms of drug-containing plasma with those of blank plasma.

Network pharmacology analysis

The identified absorbed prototype components were used as candidate active ingredients. Target prediction was performed using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (https://www.tcmsp-e.com/) with oral bioavailability ≥ 30% and drug-likeness ≥ 0.18 as screening thresholds. For components not included in TCMSP, SwissTargetPrediction (http://www.swisstargetprediction.ch) was employed for target prediction. By using "nephrotic syndrome" and "chronic kidney disease" as keywords, nephropathy-related targets were retrieved from the GeneCards (https://www.genecards.org/), OMIM (https://omim.org/), and DisGeNET (http://www.disgenet.org/) databases. The overlapping targets between component targets and disease targets were then imported into the STRING database (https://string-db.org/) to construct a protein-protein interaction (PPI) network with a confidence score ≥ 0.7 applied. The PPI network was analyzed using Cytoscape 3.9.1 (https://cytoscape.org/) to screen core targets. Gene Ontology (GO) (http://geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.kegg.jp/) pathway enrichment analyses were conducted using DAVID 6.8 (https://david.ncifcrf.gov/).

Q-Marker discovery based on chemical pattern recognition

HPLC-DAD was used to establish the fingerprint profiles of three batches of lyophilized powder. The analysis was performed on an Agilent 1260 Infinity II high-performance liquid chromatography system (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a diode array detector (DAD) for content determination and fingerprint analysis. The separation was carried out on an Agilent SB-C18 column with a mobile phase consisting of acetonitrile and 0.1% phosphoric acid in water using gradient elution. The contents of baicalin, glycyrrhizic acid, ferulic acid, and pachymic acid were determined, and their transfer rates were calculated as follows.

$$Transfer(\%) = \frac{C_{lyo} \times M_{lyo}}{\sum_{i=1}^{n}(C_{herb,i} \times W_{herb,i})} \quad (1)$$

where C_{lyo} was the concentration of the target analyte in the preparation. M_{lyo} was the total mass of the lyophilized preparation. $C_{herb,i}$ was the content of the target component in the raw medicinal material. $W_{herb,i}$ was the weight of the crude herb added. Content data of four Q-marker candidates from three batches of Xiaosiwu Decoction were subjected to Principal Component Analysis (PCA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) using SIMCA-P 14.1 software (https://www.sartorius.com/en/products/data-analytics/simca).

Statistical analysis

All statistical analyses were performed by using R 4.2.2 (https://www.r-project.org/). For multi-group comparisons, one-way analysis of variance (ANOVA) was used. When homogeneity of variance was assumed, subsequent pairwise comparisons were performed using Tukey's post-hoc test. When homogeneity of variance was not assumed, Welch's ANOVA followed by the Games-Howell test was applied. For comparisons between two independent groups, a two-tailed Student's t-test was used. The *P* values from enrichment analyses in network pharmacology were adjusted using the Benjamini-Hochberg method to control the false discovery rate. The significance of PCA and OPLS-DA models in chemical pattern recognition was evaluated by permutation tests with 200 random permutations. *P* value less than 0.05 was considered statistically significant.

Results and discussion

Identification of chemical components and blood-entry components

A total of 72 compounds were tentatively identified *via* MS/MS analysis, mainly consisting of flavonoids, terpenoids, phenylpropanoids, and organic acids. By comparison with reference standards, 12 constituents were unambiguously identified, among which baicalin, glycyrrhizic acid, ferulic acid, pachymic acid, and saikosaponin A were the predominant active components. Meanwhile, 28 prototype components absorbed into blood were detected in the administered plasma, among which baicalin, glycyrrhizic acid, ferulic acid, pachymic acid, liquiritin, wogonoside, atractylenolide III, cinnamaldehyde, and ligustilide were the main absorbed components. Saikosaponin A, saikosaponin D, and alisol A were only detected in the compound formula but not in plasma, suggesting that they might exert effects after metabolic transformation or have low bioavailability. These results indicated that absorbed components could serve as candidate active components for subsequent network pharmacology analysis.

Screening of candidate active targets in protein-protein interaction

Nephropathy associated targets were identified using the GeneCards, OMIM, and DisGeNET databases, and finally, 643 disease targets were obtained through integration and de-duplication processes. With the intersection of 485 active components targets with 643 disease targets, 112 therapeutic targets of Xiaosiwu Decoction in nephropathy were acquired. Using a degree value ≥ 18 as the screening threshold, 23 core targets were identified. The top 10 targets were

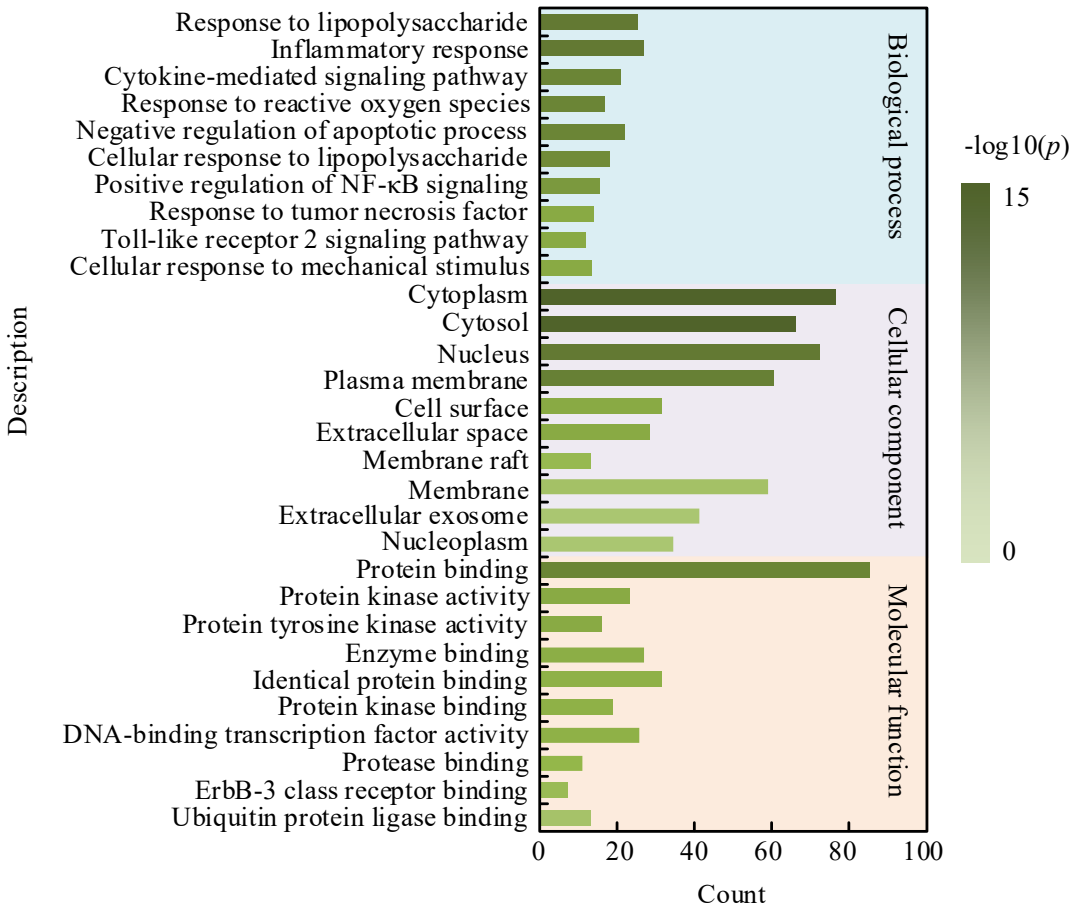


Figure 1. GO enrichment analysis results.

TNF, IL-6, VEGFA, AKT1, TP53, EGFR, SRC, MAPK3, STAT3, and JUN. These targets were mainly involved in inflammatory response, cell proliferation, and apoptosis signal transduction, suggesting that Xiaosiwu Decoction might exert its therapeutic effect on nephropathy by regulating these core targets.

Network pharmacology mechanism analysis

To explore the potential mechanism of Xiaosiwu Decoction in nephropathy, GO enrichment analysis was performed to summarize key functional associations. The results suggested that the targets were mainly involved in inflammatory regulation, oxidative stress response, and apoptosis-related processes (Figure 1). Overall, Xiaosiwu Decoction might exert renal protective effects through modulation of these biological functions. KEGG pathway enrichment analysis was performed on the intersecting targets to explore potential signaling pathways involved in the therapeutic effects of Xiaosiwu Decoction on nephropathy. The results indicated that the identified pathways were mainly related to inflammation, oxidative stress, and fibrosis (Figure 2). Overall, Xiaosiwu Decoction might exert its effects through key regulatory signaling networks associated with these pathological processes. To elucidate the interactions between regulators in terms of components, targets, and pathways, this study performed a screening of 10 components, targets, and pathways. Baicalin and glycyrrhizic acid were connected to all 10 core targets and participated in core pathways including PI3K-Akt, MAPK, AGE-RAGE, and TNF. Ferulic acid connected to 7 core targets and participated in AGE-RAGE, TNF, NF-κB, and MAPK pathways.

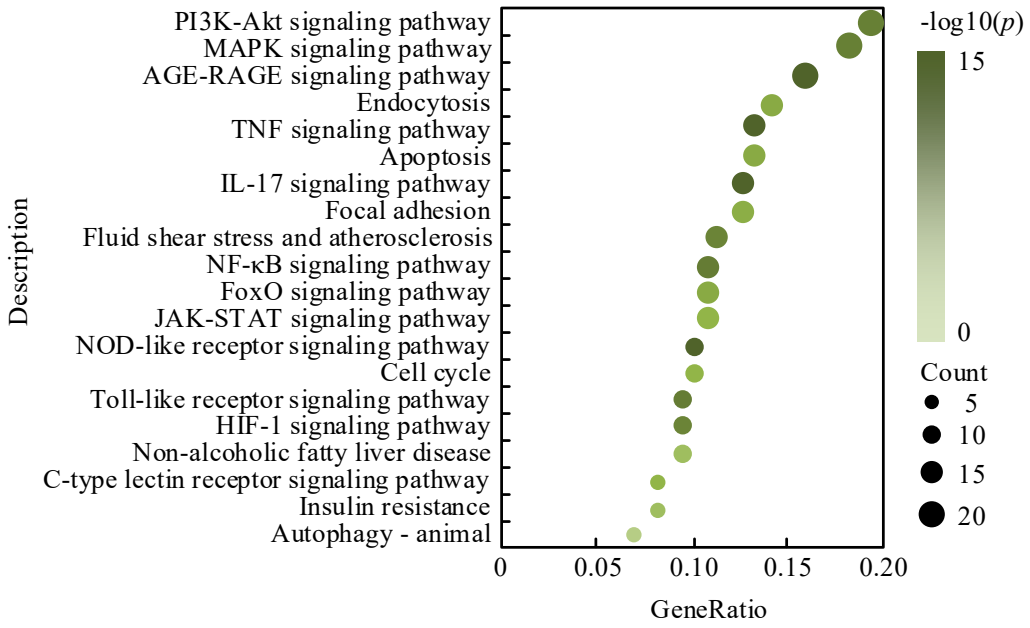


Figure 2. KEGG enrichment analysis results.

Table 1. Core component-target-pathway connection relationships.

Core component	Connected core targets	Involved core pathways
Baicalin	TNF, IL-6, AKT1, TP53, VEGFA, EGFR, SRC, MAPK3, STAT3, JUN	PI3K-Akt, MAPK, AGE-RAGE, NF-κB, TNF
Glycyrrhizic acid	TNF, IL-6, EGFR, SRC, MAPK3, STAT3, JUN, VEGFA, AKT1, TP53	PI3K-Akt, MAPK, AGE-RAGE, TNF, IL-17
Ferulic acid	TNF, IL-6, AKT1, TP53, EGFR, MAPK3, STAT3	AGE-RAGE, TNF, NF-κB, MAPK
Wogonoside	TNF, IL-6, VEGFA, AKT1, TP53, JUN	TNF, IL-17, AGE-RAGE
Liquiritin	IL-6, AKT1, EGFR, MAPK3, STAT3	PI3K-Akt, MAPK
Atractylenolide III	TNF, IL-6, AKT1, STAT3, JUN	TNF, IL-17
Ligustilide	TNF, IL-6, PTGS2, NOS2	TNF, NF-κB
Pachymic acid	AKT1, EGFR, STAT3, MAPK1	PI3K-Akt, MAPK
Cinnamaldehyde	TNF, IL-6, PTGS2	TNF, NF-κB
Poricoic acid A	AKT1, EGFR, STAT3	PI3K-Akt

Components such as wogonoside, liquiritin, and atractylenolide III were also connected to multiple core targets (Table 1). These results indicated that multiple components in Xiaosiwu Decoction acted synergistically on targets related to inflammation, proliferation, and apoptosis and coordinately regulated multiple signaling pathways.

PCA and OPLS-DA results

The content data of four candidate quality markers, namely baicalin, glycyrrhizic acid, ferulic acid and pachymic acid, in three batches of Xiaosiwu Decoction lyophilized powder were subjected to PCA and OPLS-DA analysis. The results showed that, in the PCA score plot, the cumulative variance contribution rate of the first principal component and the second principal component was 94.2%, and all three batches of samples fell within the 95% confidence interval without obvious outliers (Figure 3a). In the OPLS-

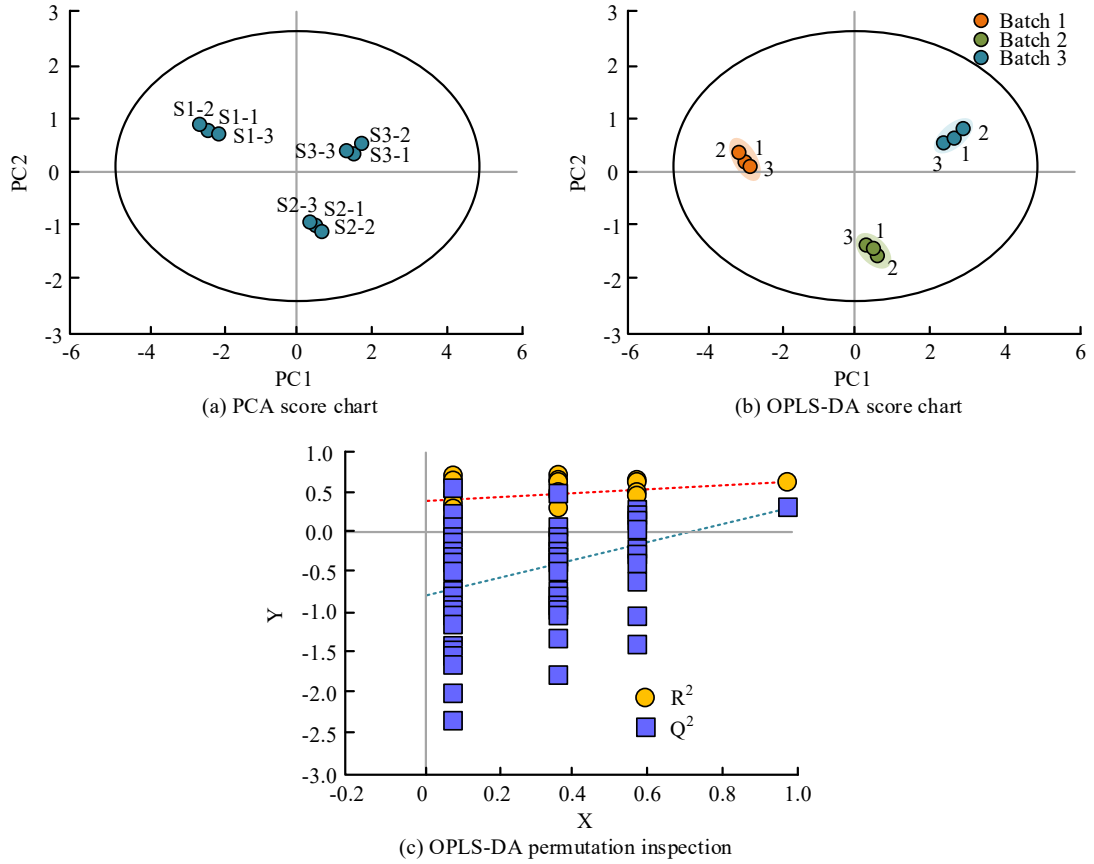


Figure 3. PCA and OPLS-DA results.

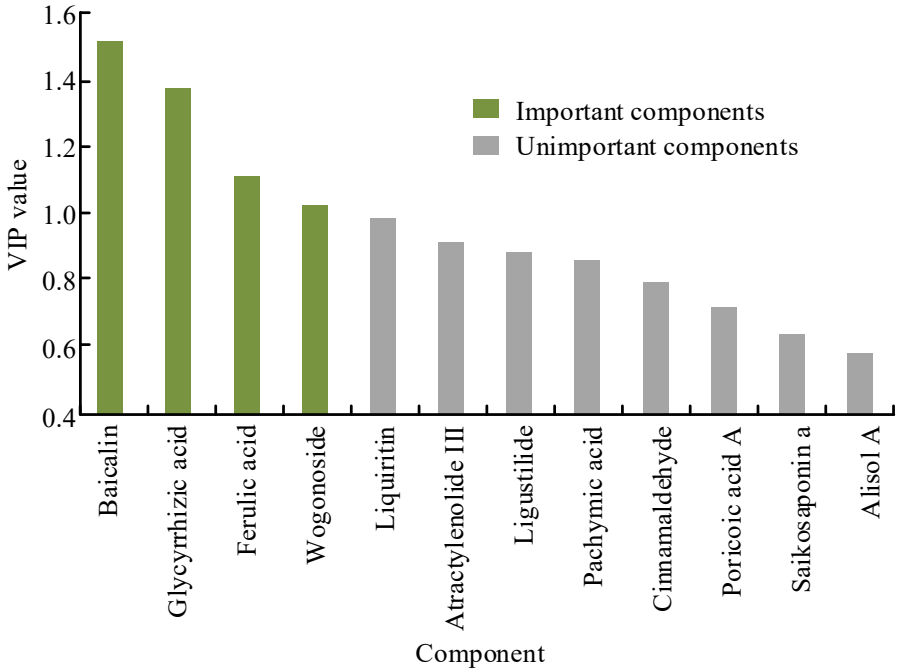


Figure 4. VIP score results.

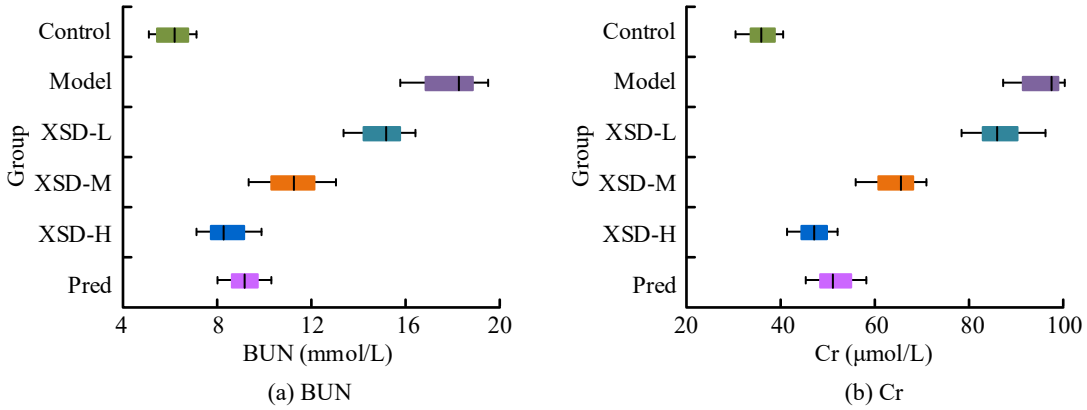


Figure 5. Serum BUN and Cr results in nephropathy animal model.

DA score plot, the three batches of samples were clearly separated along the predicted principal component direction with the analytical parameters R^2X = 0.921, R^2Y = 0.992, and Q^2 = 0.851, indicating that this method had good explanatory and predictive ability (Figure 3b). The results of the 200-permutation test indicated that all permuted Q^2 values on the left were lower than the original Q^2 value of 0.851, and the Q^2 regression line intercepted the vertical axis below zero, demonstrating the validity and robustness of the model (Figure 3c).

Pharmacological effects of Xiaosiwu Decoction on kidney disease animal model

Variable Importance in Projection (VIP) scores of the active constituents were determined using the OPLS-DA model. The results showed that the VIP scores of baicalin, glycyrrhizic acid, ferulic acid, and wogonoside were 1.52, 1.38, 1.12, and 1.05, respectively. All these VIP values exceeded 1.0, suggesting that these compounds contributed significantly to group separation. The VIP values of liquiritin, atractylenolide III, ligustilide, pachymic acid, cinnamaldehyde, poricoic acid A, saikosaponin A, and alisol A were below 1.0, indicating relatively weak contributions (Figure 4). Therefore, baicalin, glycyrrhizic acid, ferulic acid, and wogonoside could serve as preferred indicators for quality control. To evaluate the effects of Xiaosiwu Decoction on renal function in nephropathy animal model, serum BUN and Cr levels were measured across all groups. Compared with the normal control group, the model group showed markedly elevated BUN and Cr levels. Treatment with Xiaosiwu Decoction significantly reduced both parameters with the high-dose group exhibiting the most pronounced improvement (Figure 5). Overall, Xiaosiwu Decoction improved renal function in nephropathy model rats. To evaluate the effect of Xiaosiwu Decoction on inflammatory responses in nephropathy animal model, serum inflammatory factors were measured across all groups. Compared with the normal control group, the model group showed elevated levels of inflammatory markers. Treatment with Xiaosiwu Decoction reduced these indicators with more pronounced effects observed in the higher-dose group (Figure 6). Overall, Xiaosiwu Decoction effectively attenuated inflammatory responses in nephropathy model rats. To evaluate the effect of Xiaosiwu Decoction on oxidative stress in nephropathy animal model, serum oxidative stress biomarkers were measured across all groups. The model group exhibited severe oxidative stress, characterized by disrupted antioxidant biomarkers compared with the normal control group. Treatment with Xiaosiwu Decoction improved these biomarkers with more pronounced effects observed at higher doses (Figure 7). Overall, Xiaosiwu Decoction alleviated oxidative stress damage in nephropathy animal model.

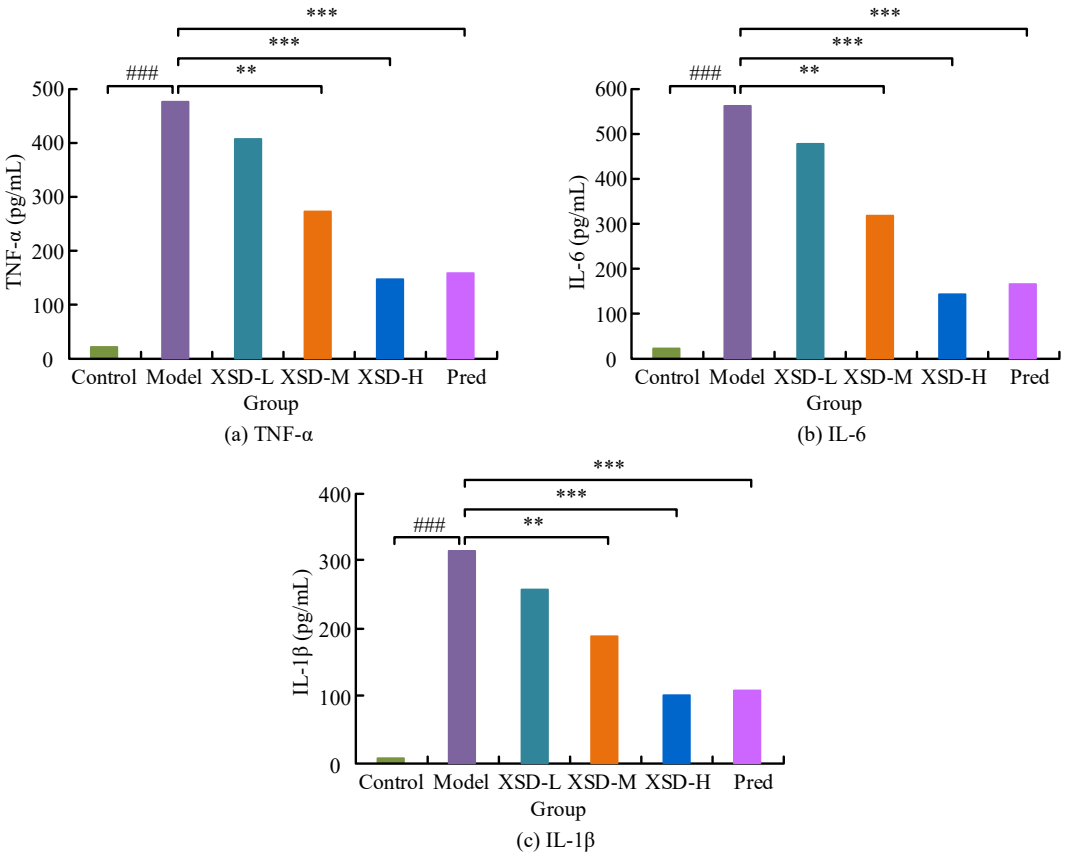


Figure 6. Serum TNF-α, IL-6, and IL-1β results in nephropathy animal model.

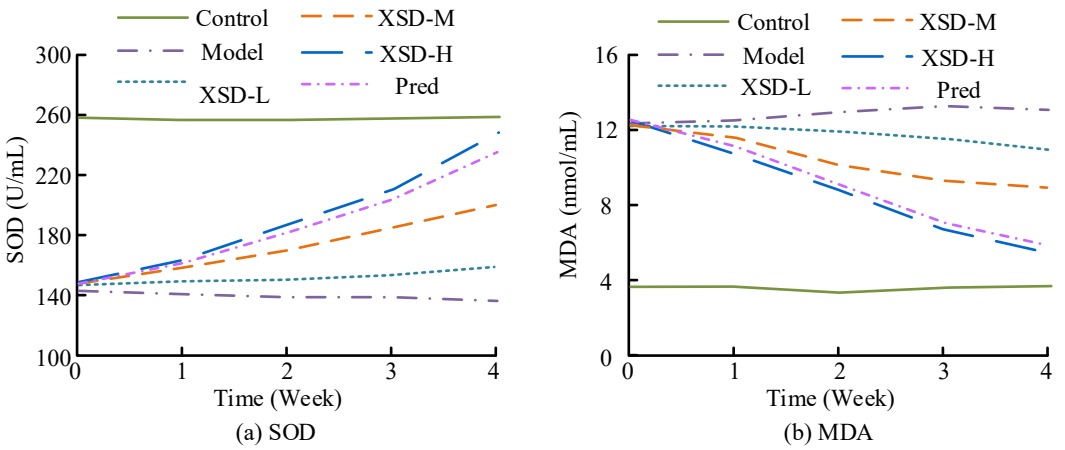


Figure 7. Serum SOD and MDA results in nephropathy animal model.

Quality control results

To verify the measurability and process transferability of the candidate quality markers, the contents of four candidate quality markers in three batches of Xiaosiwu Decoction lyophilized powder were determined by HPLC-DAD using the same preparation method. The results showed that the content differences of each component

Table 2. Q-marker content determination results.

Q-marker	Batch 1 (mg/g)	Batch 2 (mg/g)	Batch 3 (mg/g)	Mean±SD (mg/g)	RSD (%)	Transfer rate (%)
Baicalin	18.42	18.28	18.35	18.35 ± 1.02	5.6	42.3
Glycyrrhizic acid	8.81	8.69	8.78	8.76 ± 0.54	6.2	38.7
Ferulic acid	2.41	2.45	2.43	2.43 ± 0.18	7.4	51.2
Pachymic acid	1.54	1.49	1.53	1.52 ± 0.11	7.2	35.6

among the three batches were not statistically significant, indicating a stable preparation process and good batch-to-batch consistency. Baicalin showed the highest content at 18.35 ± 1.02 mg/g followed by glycyrrhizic acid at 8.76 ± 0.54 mg/g, ferulic acid at 2.43 ± 0.18 mg/g, and pachymic acid at 1.52 ± 0.11 mg/g. The inter-batch relative standard deviation (RSD) was less than 6% for all markers, indicating high stability of the preparation process. The transfer rates for baicalin, glycyrrhizic acid, ferulic acid, and pachymic acid were determined to be 42.3%, 38.7%, 51.2%, and 35.6%, respectively (Table 2). It was evident that all these compounds remained intact throughout the decoction and concentration process and could be quantified as parent compounds after absorption in blood. These data suggested that baicalin, glycyrrhizic acid, ferulic acid, and pachymic acid satisfied the measurability and traceability criteria for Q-markers and hence could be considered potential candidates for Q-markers of Xiaosiwu Decoction.

Conclusion

To identify Q-markers for Xiaosiwu Decoction and establish a robust quality control system, this study integrated network pharmacology with multi-omics technology to investigate the chemical profile and *in vivo* metabolic regulation of Xiaosiwu Decoction. This study identified 72 chemical constituents and 28 absorbed prototype compounds in Xiaosiwu Decoction using UPLC-Q-TOF-MS/MS, thereby clarifying the material basis of the formula. Network pharmacology revealed the associations of core targets such as TNF, IL-6, and AKT1 with PI3K-Akt, MAPK, and inflammatory pathways. Meanwhile, animal experiments verified the pharmacodynamic effects of Xiaosiwu Decoction in improving renal function, inhibiting inflammation, and regulating oxidative stress. Chemical pattern recognition and content determination further confirmed the batch discrimination ability and process transferability of the candidate components. The multi-dimensional quality control system constructed in this study provided a scientific basis for establishing quality standards of Xiaosiwu Decoction. While the current study combined omics and network pharmacology to determine the Q-markers of Xiaosiwu Decoction, the direct relationship between these Q-markers and therapeutic effects could not be verified using clinical samples. Further studies should be conducted to validate these findings in clinical cohorts and through metabolic enzyme intervention assays.

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