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Original Article

Exploring the therapeutic effect of core components in Xuanshen Yishen mixture on hypertension through network pharmacology

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Abstract: Objective: This study aims to elucidate the mechanism of action and impact of the "Xuanshen Yishen Mixture" (XYM) on hypertension. Methods: Active components were identified and potential targets were predicted using the Traditional Chinese Medicine Systems Pharmacology database. Hypertension-related targets were collected from GeneCards, DRUGBANK, OMIM, TTD, and PharmaGKB databases. Intersections of disease and drug targets were visualized using the R package "VennDiagram". A protein-protein interaction network was established via the STRING database. GO function enrichment and KEGG pathway analyses were conducted using "clusterProfiler", while "Cytoscape" was used to construct a "drug-component-target" network. Additionally, data from 60 patients with essential hypertension from the Affiliated Hospital of Shandong University of Traditional Chinese Medicine were retrospectively analyzed. Patients were divided into a control group (n = 30) and an XYM group (n = 30) based on treatment regimen. Results: Sixty active ingredients and 98 related targets were identified from Uncaria, Radix Scrophulariae, and Epimedium in hypertension treatment. Key active components such as quercetin, kaempferol, yohimbine, and beta-sitosterol were pinpointed, with PTGS2, PTGS1, AR, DPP4, and F2 as crucial targets. KEGG pathway analysis highlighted significant pathways including IL-17 signaling, TNF signaling, Relaxin signaling, and HIF-1 signaling. Clinical data indicated that XYM's therapeutic effects are comparable to those of valsartan, which significantly reduced diastolic and systolic blood pressure and demonstrated good biosafety. Conclusions: Uncaria, Radix Scrophulariae, and Epimedium effectively mitigate hypertension through multiple components, targets, and pathways. Additionally, DPP4, IL-17, and TNF-α are identified as potential therapeutic targets for traditional Chinese medicine preparations in hypertension treatment. This study provides a foundation for further investigation into XYM's mechanisms in hypertension management.

Keywords: Xuanshen Yishen mixture, network pharmacology, Uncaria, Radix Scrophulariae, Epimedium, hypertension

Introduction

Essential Hypertension, commonly referred to as hypertension, is one of the most prevalent chronic cardiovascular diseases globally and represents a significant public health challenge. Epidemiological studies predict a 26.4% to 60% increase in the global hypertensive population by 2025, potentially affecting up to 1.56 billion individuals [1]. In China, approximately 300 million people suffer from hypertension [2], with the prevalence rate continuing to rise. Currently, about 23% of the Chinese population is afflicted with this condition, placing considerable burden on both families and societal resources. Thus, the development of effective intervention drugs for hypertension is of paramount medical and social importance.

Traditional Chinese Medicine (TCM) has been practiced for over 2000 years and is renowned for its holistic approach, particularly its emphasis on "syndrome differentiation and treatment". Recent advancements in the research on the etiology, pathogenesis, and clinical treatment of hypertension have yielded promising

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results. The TCM theory of "deficiency of yin and yang" is considered a crucial pathological factor in hypertension [3]. National Guidelines for Management of Hypertension Control at the Primary Level 2020 Edition [4] recently incorporated TCM into the official management protocols for primary hypertension, reflecting its growing significance in clinical practice.

Xuanshen Yishen Mixture (XYM), a proprietary blend developed by the Affiliated Hospital of Shandong University of Traditional Chinese Medicine, is formulated to nourish yin and yang. Comprising primarily Radix Scrophulariae, Uncaria, and Epimedium, XYM has demonstrated potential benefits in managing hypertension, though yet identified mechanisms. In particular, the potential targets, biological processes, and metabolic pathways of XYM remain largely unexplored. Network pharmacology, which accounts for the synergistic effects of multi-component, multi-channel, and multi-target interventions, is ideally suited for analyzing TCM formulations [5, 6]. This study aims to dissect the active components, key targets, and pathways of Uncaria, Radix Scrophulariae, and Epimedium in treating hypertension using network pharmacology.

Materials and methods

Screening of drug-related targets

The Traditional Chinese Medicine Systems Pharmacology database and analysis platform (http://tcmspw.com/tcmsp.php) [7] was utilized to identify components and related targets of Uncaria, Radix Scrophulariae, and Epimedium. Screening for Absorption, Distribution, Metabolism, and elimination properties was conducted, with active components and drug targets initially identified based on oral bioavailability ≥ 30% and drug-likeness ≥ 0.18. The effective components and targets were then standardized using the Uniprot database [8].

Acquisition of hypertension-related targets

Disease targets were searched using the keyword "hypertension" in the GeneCards, DRUGBANK, OMIM, TTD, and PharmGKB databases [9-13]. The screening criterion for disease targets in GeneCards was a relevance score > 5. The targets from all five databases were merged, and duplicates were removed.

Acquisition of potential targets for drug treatment of diseases

The R package "VennDiagram" was used to identify the intersections between drug targets and disease targets, highlighting the potential targets of XYM against hypertension [14].

Protein-protein interaction (PPI) network construction

Potential drug targets were uploaded to the STRING database [15] (https://cn.string-db.org/), and a PPI network was constructed with a medium confidence score threshold of ≥ 0.4. The network was visualized using Cytoscape (version 3.7.2).

Enrichment analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted using the R package "clusterProfiler" [16]. The GO analysis encompassed Biological processes, molecular functions, and cellular components. Terms and pathways with an adjusted *p*-value (adj.p) < 0.05 were deemed statistically significant. The top 5 GO terms and the top 10 KEGG pathways were selected for visualization.

Network construction of "drug component target"

The software Cytoscape was utilized to construct a "drug-component-target" network for the treatment of hypertension using "Uncaria-Scrophularia-Epimedium". Nodes in the network represented the drugs, components, and targets, while edges depicted their relationships. Network Analyzer was employed to analyze network characteristics, including Degree, Betweenness, and Closeness, allowing for the identification of core targets and main active components based on network topology parameters.

Molecular docking evaluation

The main active components and core targets were validated through molecular docking. Compound structures were evaluated using the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [17]. Core proteins were retrieved from the RCSB Protein Data Bank database (https://www.rcsb.org/). PyMOL soft-

ware (http://www.pymol.org) and AutoDockTools (http://autodock.scripps.edu/resources/tools) facilitated protein dehydration, hydrogenation, and other preparatory steps. Compound and target protein file formats were converted to pdbqt using AutoDock Vina [18] (http://vina.scripps.edu/), and molecular docking was performed. The active pocket was identified, and docking results were obtained, with those showing a docking value affinity < -4.25 kcal/mol considered indicative of binding activity. Scores < -5.0 kcal/mol suggested better binding activity, and scores < -7.0 kcal/mol indicated strong binding activity. Molecular docking results were visualized using PyMOL software.

Ethic statement

Research experiments in this article were approved by the Ethical Committee of the Affiliated Hospital of Shandong University of Traditional Chinese Medicine.

Clinical case selection

In this study, data from 60 patients with essential hypertension from the Affiliated Hospital of Shandong University of Traditional Chinese Medicine were retrospectively analyzed. Inclusion criteria: All patients met the diagnostic criteria [4] for essential hypertension, classified as grade 1; patients had been treated with either Xuanshen Yishen mixture or valsartan for 4 weeks; and all clinical data were complete. Exclusion criteria: Patients with secondary hypertension or other serious organ diseases were excluded.

Therapeutic methods

Patients were divided into two groups based on the treatment received: the control group (n = 30) treated with valsartan capsules (Beijing Novartis Pharmaceutical Co., Ltd., Daiwen) at a dose of one tablet per day (80 mg/day), with each treatment course lasting 4 weeks and comprising one consecutive; and the XYM group (n = 30) treated with Xuanshen Yishen mixture, 30 mL tid, with each treatment course also lasting 4 weeks and comprising one consecutive. The XYM, prepared by the hospital, included Uncaria, Radix Scrophulariae, and Epimedium. Data were collected from patients before and after treatment, including gender, age, duration of disease, blood pressure, blood lipid levels, and laboratory indicators (aspartic transaminase, alanine transaminase, serum creatinine, blood leukocytes, hemoglobin).

Statistical analysis

Data were processed using SPSS version 21.0 software. Quantitative data consistent with a normal distribution were expressed as mean ± SD (n = 9). Between-group comparisons were performed using the t-test, and among-group comparisons were conducted using one-way analysis of variance followed by Tukey's test. A *P* value of < 0.05 was considered statistically significant.

Results

Screening and target prediction of the active ingredients of Uncaria, Radix Scrophulariae, and Epimedium

Analysis revealed 60 active ingredients, with one attributed to a combination of Uncaria, Radix Scrophulariae, and Epimedium, another specific to Uncaria and Radix Scrophulariae, and two shared between Uncaria and Epimedium. Individually, 29 active ingredients were linked to Uncaria, 7 to Radix Scrophulariae, and 20 to Epimedium. After combining the target prediction results and removing duplicates, a total of 215 targets were identified, as detailed in **Table 1**.

Acquisition of disease targets

From the GeneCards database, 10,684 potential disease targets were initially identified. Only those with a Relevance Score greater than 5 were selected as pertinent disease targets. This process was replicated with the TTD, OMIM, DRUGBANK, and PharmGKB databases, with duplicates subsequently removed, culminating in a refined list of 901 targets (Supplementary Appendix 1).

Potential targets of Uncaria, Radix Scrophulariae, and Epimedium in hypertension treatment

The 901 disease targets of hypertension were intersected with the 215 drug targets of "Uncaria Uncaria Scrophulariae Epimedium", revealing a total of 98 common targets, as shown in **Figure 1**.

Table 1. Network topology parameters of key active ingredient

MolID	Name	Degree	Betweenness centrality	Closeness centrality	Herb
MOL000098	Quercetin	78	0.577571	0.598394	URC
MOL000422	Kaempferol	35	0.101539	0.447447	URC EH
MOL008488	Yohimbine	18	0.062179	0.405995	URC
MOL000358	Beta-sitosterol	17	0.042593	0.408219	URC FR
MOL004391	8-(3-methylbut-2-enyl)-2-phenyl-chromone	16	0.018847	0.403794	EH

Note: URC, Uncaria e Ramulus Cumulus (Uncaria); FR, Figwort Root (Radix Scrophulariae); EH, Epimedii Herba (Epimedium).

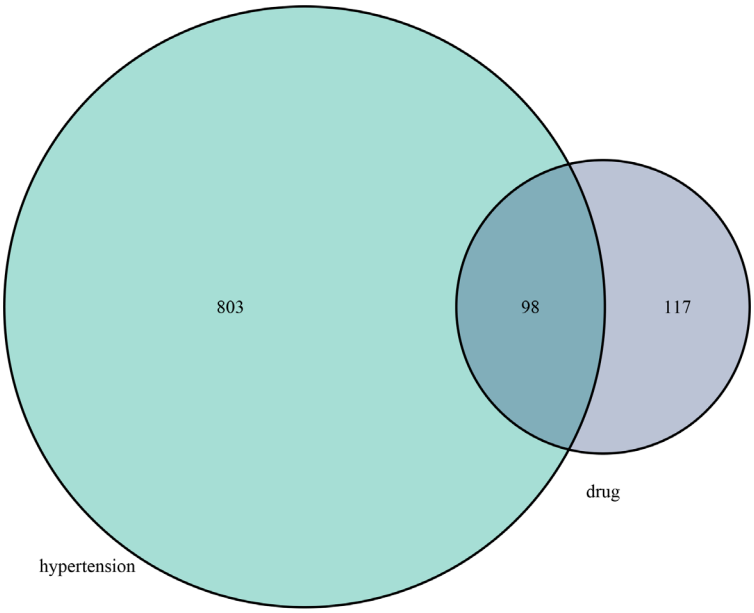

Figure 1. Venn diagram of targets of Xuanshen Yishen Mixture and hypertension.

PPI network construction

The 98 intersecting targets were uploaded to the STRING database, resulting in a PPI network comprising 98 nodes and 1,355 edges. The nodes represent the intersection targets, while the edges denote the interactions between these targets. Cytoscape software facilitated the visual analysis of this PPI network (**Figure 2**), and the Network Analyzer function was employed to calculate the degree value of the nodes. Nodes with higher degree values were considered more central and potentially more significant within the network.

GO function enrichment and KEGG pathway analyses

GO function enrichment and KEGG pathway analyses were performed on 98 potential targets of Uncaria, Radix Scrophulariae, and Epimedium in the treatment of hypertension. Biological process results primarily involved responses to xenobiotic stimuli, wound healing, processes affecting the circulatory system, metabolic processes involving reactive oxygen species, and regulation of tube diameter. Cellular component findings predominantly included membrane raft, membrane microdomain, caveola, plasma membrane raft, and endocytic vesicle. Molecular function results largely featured heme binding, nuclear receptor activity, ligand-activated transcription factor activity, tetrapyrrole binding, and receptor-ligand activity (**Figure 3**). The KEGG pathway analysis highlighted enrichment in pathways such as IL-17 signaling, TNF signaling, relaxin signaling, HIF-1 signaling, and pathways related to lipid metabolism and atherosclerosis (**Figure 3**).

Drug component target network

The network was analyzed using Cytoscape, consisting of 150 nodes and 502 edges. In **Figure 4**, the drug is represented as a diamond node, active ingredients as V-shaped nodes, and potential targets as circular nodes. The node size of potential targets indicates their degree, reflecting their importance in the network. Higher degree nodes are larger and more significant. Each active ingredient corresponds to multiple targets, demonstrating the multi-target actions of Uncaria, Radix Scrophulariae, and Epimedium in hypertension treatment. The top 5 components and targets, based on net-

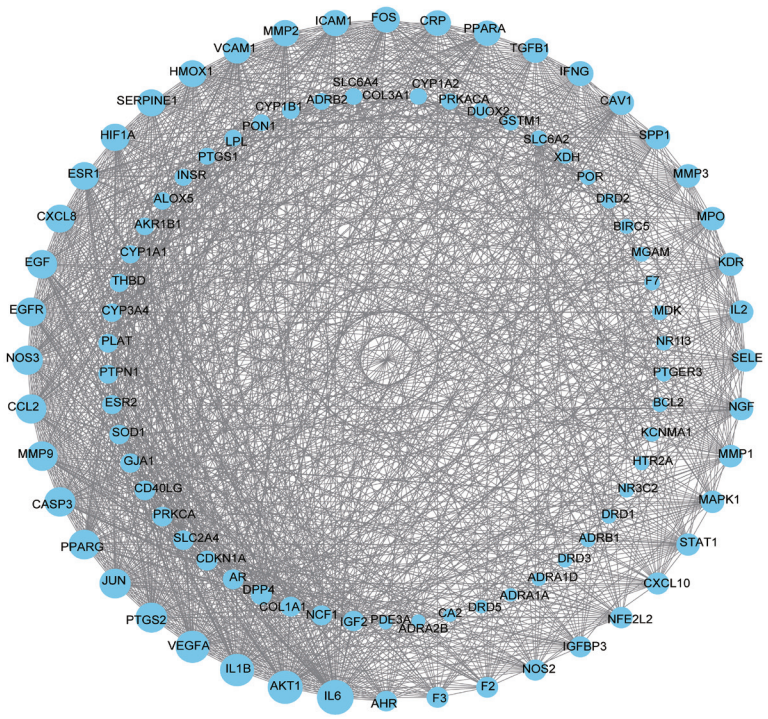

Figure 2. PPI network of common targets between Xuanshen Yishen Mixture and hypertension.

work topology parameters, are detailed in **Tables 2** and **3**.

Molecular docking evaluation

The binding activity between the target proteins and the active components was assessed through molecular docking. The top two active components, quercetin and kaempferol, were docked with the four key targets: PTGS2, PTGS1, AR, and DPP4. Eight sets of receptor-ligand docking results were obtained, as depicted in **Figure 5**. The receptor-ligand affinity scores for all groups were below -5.0 kcal/mol, indicating strong binding activity between the key active components and their targets.

Evaluation of antihypertensive effect of XYM

A total of 60 patients with essential hypertension participated in this study. No significant differences were observed in age, sex, duration of disease, blood pressure, cholesterol, or other lipid levels between the two groups (all $P > 0.05$) (**Table 4**). After one month of antihypertensive treatment, both systolic blood pressure and diastolic blood pressure significantly decreased, with no significant differences between the groups post-treatment (**Figures 6** and **7**). Laboratory indices including aspartate transaminase, alanine transaminase, serum creatinine, white blood count, and hemoglobin levels remained unchanged pre- and post-treatment, and no adverse drug reactions such as nausea, vomiting, or allergies were reported, indicating the treatments are safe and effective.

Discussion

Hypertension is a chronic disease contributing significantly to the global disease burden [19]. Often asymptomatic, prolonged hypertension can lead to damage in multiple target organs, such as the heart, brain, and kidneys. According to the China Hypertension Survey [20], the prevalence of hypertension in adults over 18 years old in China is 27.9% [21], with a higher proportion in males and an increase with age. In TCM, hypertension is commonly treated based on symptoms like dizziness, a term historically associated with the disease. The concept of “liver wind”, as first described in “Su Wen Feng Lun”, correlates closely with the clinical manifestations of hypertension.

In TCM, compatibility of herbs is the foundational of prescription formulations. TCM compounds aim to enhance efficacy, reduce toxicity, and improve therapeutic outcomes by integrating multiple herbs. Given the complex composition and unidentified targets of Chinese medicines, network pharmacology offers a valuable approach. It allows for a comprehensive analysis of the components and targets of TCM, constructing networks of active components and targets that visually demonstrate the multi-target and multi-channel synergistic advantages of TCM. Although XYM is frequently used for treating hypertension, its mechanism remains poorly understood.

Our network pharmacology results identified 15 compounds in Uncaria, Radix Scrophulariae, and Epimedium, which target 23 key signaling molecules associated with hypertension. These

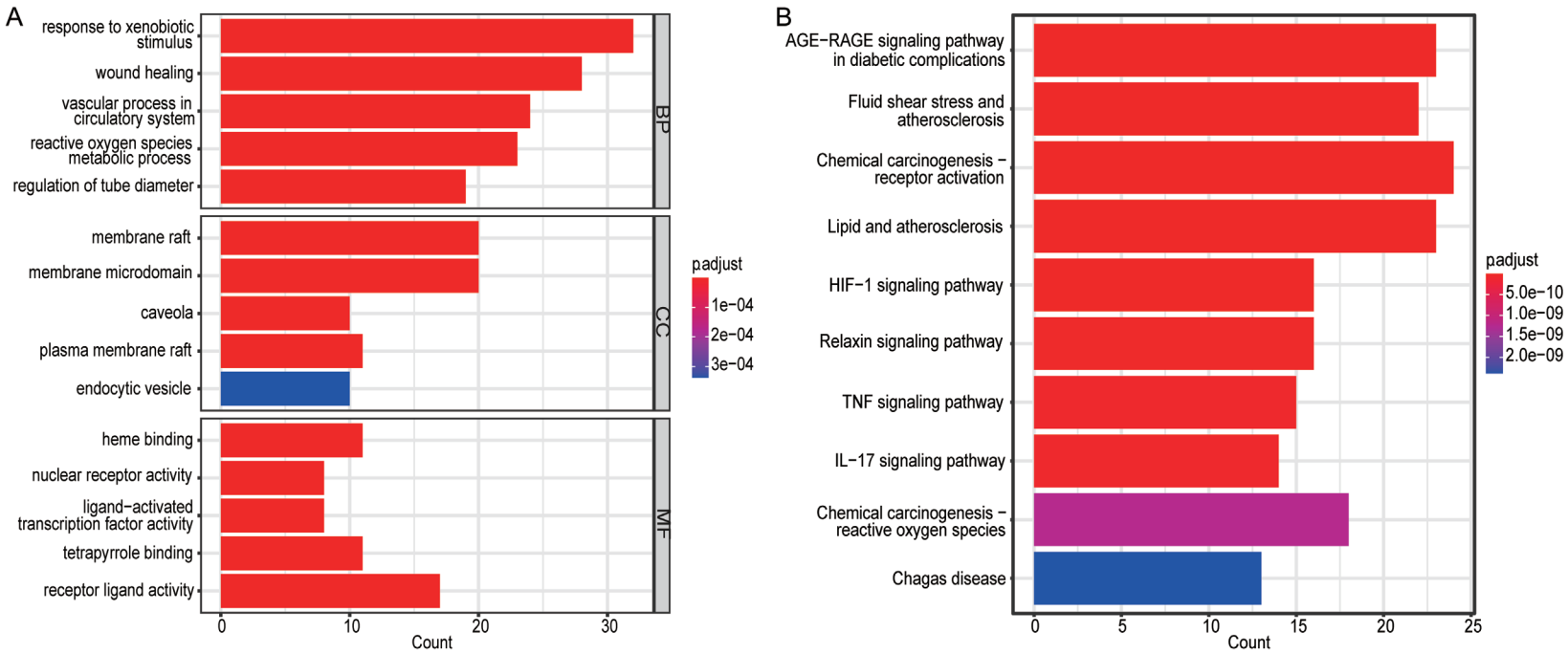

Figure 3. GO function and KEGG pathway enrichment analysis diagram. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes. A: GO function analysis diagram. B: KEGG pathway enrichment analysis diagram.

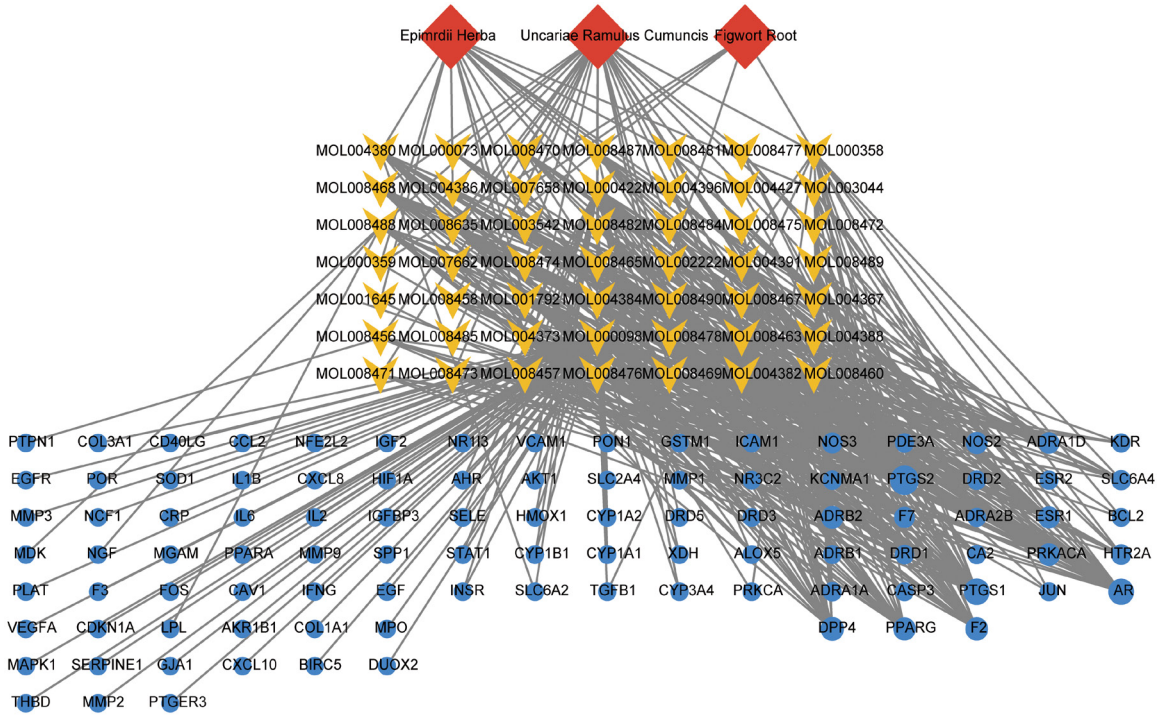

Figure 4. "Drug Component Target" network diagram.

Table 2. Network topology parameters of key active ingredients

MolID	Name	Degree	Betweenness centrality	Closeness centrality	Herb
MOL000098	Quercetin	78	0.577571	0.598394	URC
MOL000422	Kaempferol	35	0.101539	0.447447	URC EH
MOL008488	Yohimbine	18	0.062179	0.405995	URC
MOL000358	Beta-sitosterol	17	0.042593	0.408219	URC FR
MOL004391	8-(3-methylbut-2-enyl)-2-phenyl-chromone	16	0.018847	0.403794	EH

Note: URC, Uncaria e Ramulus Cumulus (Uncaria); FR, Figwort Root (Radix Scrophulariae); EH, Epimedii Herba (Epimedium).

Table 3. Key target network topology parameters

Name	Degree	Betweenness centrality	Closeness centrality
PTGS2	46	0.161528	0.579767
PTGS1	34	0.086868	0.530249
AR	33	0.0704	0.515571
DPP4	23	0.036312	0.467085
F2	21	0.030557	0.4613

include PTGS2, PTGS1, AR, DPP4, and F2, which play significant roles in pathways such as IL-17, TNF, Relaxin, and HIF-1 signaling. Four main compounds - quercetin, kaempferol, yohimbine, and beta-sitosterol - were linked to cardiovascular benefits. Quercetin, a low molecular weight polyphenol found in Uncaria, has been extensively studied for its antihypertensive and cardiovascular protective effects [22]. It inhibits the renin-angiotensin-aldosterone system, and affects vascular smooth muscle cell contraction [23-26]. In experimental models with spontaneously hypertensive rats, quercetin not only reduced the F/B ratio but also improved gut microbiota diversity [23-26]. Kaempferol, present in both Uncaria and Epimedium, has been shown to promote nitric oxide release in endothelial cells and counter-

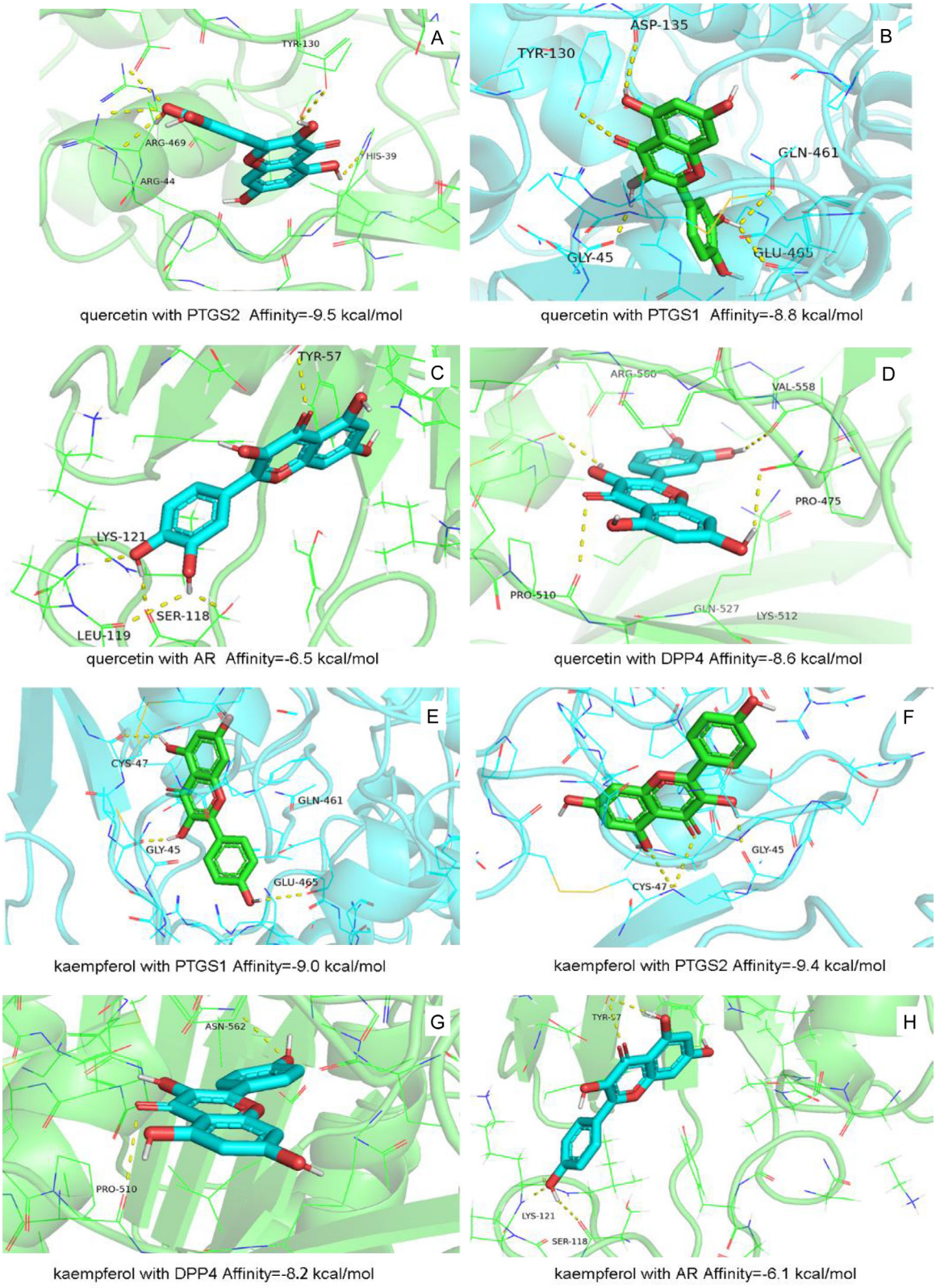

Figure 5. Molecular docking modes of some important components and key targets. A. Quercetin with PTGS2 Affinity = -9.5 kcal/mol. B. Quercetin with PTGS1 Affinity = -8.8 kcal/mol. C. Quercetin with AR Affinity = -6.5 kcal/mol. D. Quercetin with DPP4 Affinity = -8.6 kcal/mol. E. Kaempferol with PTGS1 Affinity = -9.0 kcal/mol. F. Kaempferol with PTGS2 Affinity = -9.4 kcal/mol. G. Kaempferol with DPP4 Affinity = -8.2 kcal/mol. H. Kaempferol with AR Affinity = -6.1 kcal/mol.

Table 4. Comparison of basic data of hypertensive patients

	Control group (n = 30)	XYM group (n = 30)	P
Gender (Male/female)	13/17	18/12	0.196
Age (year)	54.3±8.33	52.47±10.36	0.453
DBP (mmHg)	92.23±4.33	93.53±4.3	0.085
SBP (mmHg)	146.23±5.99	148.53±5.9	0.389

Note: DBP, diastolic blood pressure; SBP, systolic pressure.

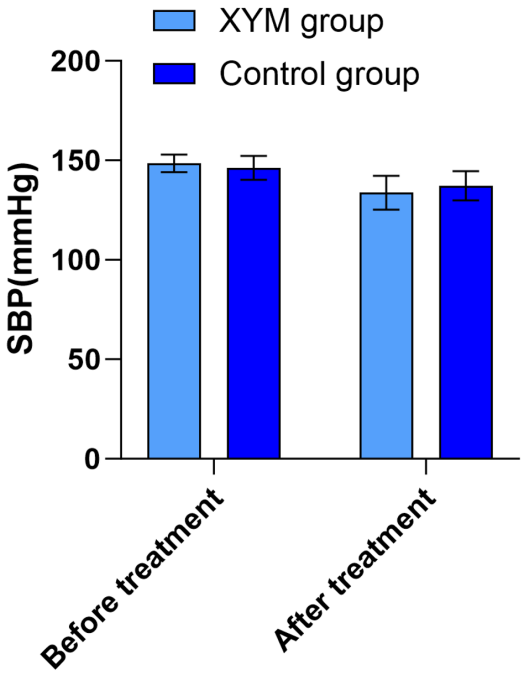

Figure 6. Comparison of systolic blood pressure (SBP) before and after treatment.

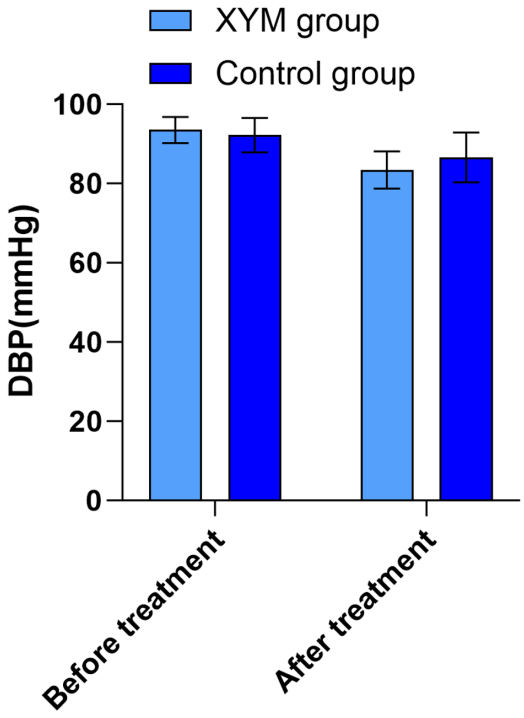

Figure 7. Comparison of diastolic blood pressure (DBP) before and after treatment.

act calcium channels in vascular smooth muscle, thus lowering blood pressure [27-31]. Yohimbine, primarily found in Uncaria, is a natural indole alkaloid that acts as an effective α2-adrenergic receptor antagonist, dilating peripheral blood vessels and reducing blood pressure [32, 33]. Beta-sitosterol, identified in both Uncaria and other TCM herbs, exhibits diverse health benefits, including anti-inflammatory, antioxidant, anti-atherosclerotic, lipid-lowering, antihypertensive, and target organ protective effects [34-39].

This study identified PTGS1, PTGS2, AR, and DPP4 as crucial targets for hypertension management using XYM. PTGS1 and PTGS2 may influence hypertension through their roles in prostaglandin metabolism and their involvement in oxidative stress and inflammatory reactions [40, 41]. The inhibition of Cox-2 has shown potential in reducing myocardial hypertrophy and fibrosis in hypertensive mice [42]. AR, being transmembrane glycoproteins linked to G-proteins, may impact blood pressure by mediating the sympathetic adrenal response to stress [43]. DPP4, a serine protease, influences blood pressure through its soluble form, sDPP4, which can induce vascular smooth muscle proliferation, inflammation, and endothelial dysfunction [44, 45].

Network pharmacology analysis revealed that Uncaria, Radix Scrophulariae, and Epimedium primarily modulate hypertension via the IL-17 signaling pathway, TNF signaling pathway, relaxin signaling pathway, and HIF-1 signaling pathway. Elevated blood flow shear stress is known to maintain normal endothelial function, reduce the secretion of endothelial injury fac-

tors, protect the vascular intima, and effectively prevent hypertension [46]. TNF is critical for vascular endothelial injury and the proliferation of vascular smooth muscle cells, influencing the expression and release of IL-6, thus playing a significant role in hypertension development [47]. IL-17 overexpression can induce systemic endothelial dysfunction and hypertension [48]. Relaxin, a polypeptide hormone, protects cardiovascular and cerebrovascular systems through mechanisms including endothelial protection, anti-fibrosis, anti-inflammation, antioxidant activity, and vascular relaxation. It significantly reduces blood pressure in various hypertensive models [49]. HIF-1, activated under hypoxic conditions, increases gene expression related to cell growth, proliferation, migration, and apoptosis, thereby affecting genes regulating blood viscosity and vascular endothelial function [50-52]. These pathways are implicated in the pathogenesis of hypertension.

Clinical data analysis indicates that the Xuanshen Yishen mixture has a therapeutic effect comparable to that of valsartan capsules in treating hypertension. Both treatments demonstrated significant antihypertensive effects without notable adverse drug reactions.

The study provided insights into XYM's potential mechanism in treating hypertension, predicting key targets, and elucidating the roles of core compounds and crucial genes in relevant pathways. These findings not only enhance understanding of XYM's therapeutic effects but also establish a methodological framework for investigating the pharmacological mechanisms of TCM. Future research should systematically explore hypertension treatment with XYM to delineate its specific mechanisms experimentally.

Although this study offers valuable data and a theoretical basis for further screening and evaluation of antihypertensive TCM ingredients, some compounds previously identified as therapeutically effective in the literature were missing, indicating the limitations of the experimental approach. Therefore, the results require further verification and refinement.

Acknowledgements

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Disclosure of conflict of interest

None.

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