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A multi-strategy platform for Q-markers screening and quality control of Wuzi Yanzong Wan based on fingerprint and network pharmacology

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ABSTRACT

Wuzi Yanzong Wan (WZYZW) is a classic formula for tonifying the kidneys in Traditional chinese medicine (TCM) and is widely used clinically in diseases of the reproductive system, immune system and nervous system. Therefore, the quality control and prediction quality markers (Q-markers) of WZYZW is of positive significance. In this study, We first established the fingerprints, calibrated 28 common peaks, identified 12 common peaks of gallic acid, geniposidic acid, chlorogenic acid, ellagic acid, rutin, hyperoside, verbascoside, astragalin, kaempferol, schisandrol A, schisandrol B and schisandrin B, and the similarity was greater than 0.9 of 10 batches.Then, withing network pharmacology, we screened 41 core targets such as PIK3CA, AKT1, PIK3CB, potential pathways such as pathways in cancer and PI3K-Akt signalling pathway of 12 components. The results showed that they were tightly connected to predicated targets and pathways. Finally, by using the hyperoside as internal reference standard, a Quantitative analysis of multi-components (QAMS) for WZYZW was established to determine the contents of WZYZW. The percent difference(%) showed that there was no significant difference between the quantitative results of external standard method (ESM) and QAMS method. Contents of the 11 components above were 0.0091 ~ 0.0185 %, 0.1019 ~ 0.1361 %, 0.0518 ~ 0.0718 %, 0.0130 ~ 0.0320 %, 0.0084 ~ 0.0190 %, 0.0621 ~ 0.1192 %, 0.0127 ~ 0.0419 %, 0.0006 ~ 0.0089 %, 0.0212 ~ 0.0261 %, 0.0061 ~ 0.0400 %, 0.0084 ~ 0.0103 %, respectively. Overall, the method developed was applied to the quality evaluation and Q-markers research of WZYZW as well as provided guiding significance for the Q-marker research of TCM.

1. Introduction

WZYZW is a traditional TCM formula for tonifying the kidney and essence. It is a combination of *cuscutae semen* (*Cuscuta australis* R.Br. or *Cuscuta chinensis* Lam., chinese name Tusizi), *Lycii fructus* (*Lycium barbarum* L., chinese name Gouqizi), *plantaginis semen* (*Plantago asiatica* L. or *Plantago depressa* Willd., chinese name Cheqianzi), *rubi fructus* (*Rubus chingii* Hu,chinese name Fupengzi), and *schisandrae chinensis fructus* [*Schisandra chinensis* (Turcz.) Baill., chinese name Wuweizi]. Because of its effects of tonifying the essence, inducing kidney qi and reproduction, WZYZW has been respected by generations of doctors, being called "The Number One Herbal Formula for Assisting Fertility" (Chinese Pharmacopoeia Commission, 2020). In this formula, *Lycii fructus* warms the

Abbreviations: WZYZW, Wuzi Yanzong Wan; TCM, Traditional chinese medicine; Q-markers, Quality markers; QAMS, Quantitative analysis of multi-components; PPI, Protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; SA, Similarity analysis; ESM, External standard method; HPLC, High Performance Liquid Chromatography; BP, Biological processes; MF, Molecular functions; CC, Cellular components; PIK3CA, Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; PIK3CB, Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta; RTK, Receptor tyrosine kinase; PI3K, Phosphatidylinositide 3-kinases; PIP3, Phosphatidyl-inositol,3,4,5 triphosphate; GPCR, G protein-coupled receptor; EGFR, Epidermal growth factor receptor; RSD, Relative standard deviation; RCF, Relative Correction Factors; PD, Percentage difference; Ch.P, Chinese Pharmacopoeia; TCMSP, Traditional Chinese Medicine Systems Pharmacology; GAP43, Growthassociated protein 43; AD, Alzheimer's disease; DIC, Diclofenac; BACE1, β-site APP-cleaving enzyme; HCC, Hepatocellular carcinoma; BMP-7, Bone morphogenetic protein 7; ATP, Adenosine-triphosphate.

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Nomenclature

fsk=Wk×ASWs×Ak

As	the peak area of the internal component "s"
Ws	the concentration of the internal component "s"
Ak	the peak area of the target component "k"
Wk	the concentration of the target component "k"
H	*H* stands for Hyperoside
G1	*G1* stands for Gallic acid
G2	*G2* stands for Geniposidic acid
C	*C* stands for Chlorogenic acid
E	*E* stands for Ellagic acid
R	*R* stands for Rutin
V	*V* stands for Verbascoside
A	*A* stands for Astragalin
I	*I* stands for Kaempferol
S	*S* stands for Schisandrol A
D	*D* stands for Schisandrol B
K	*K* stands for Schisandrin B

ris=tR(s)tR(i)

$t_{R(i)}$	the retention time of the component to be measured
$t_{R(s)}$	the retention time of the reference component.

PD=(QAMS-ESM)(QAMS+ESM)2×100%

QAMS	QAMS stands for the content of the component measured by QAMS method
ESM	ESM stands for the content of the component measured by ESM method

kidney and enhances the essence, and *rubi fructus* nourishes the kidney and fills the essence, the two drugs supplement yin and yang together, and are the monarch; *schisandrae chinensis fructus* invigorates qi、strengthens yin and astringent essence, *cuscutae semen* warms the kidneys without drying, solidifying the essence but not coagulation, and are the minister; *plantaginis semen* is both astringent, tonic but not stagnant, used as an assistant medicine (Li et al., 2019), Clinically, it has an extensive range of applications in reproductive system and nervous system diseases.

WZYZW is composed of multiple herbs, the compatibility and composition of medicinal flavors are more complex, and the quality of decoction pieces is also uneven. At present, there are few researches on the integrated quality control of WZYZW. As a consequence of that, it is of great significance to develop a method that can comprehensively explain the characteristics of WZYZW compounds. Based on this background, Academician Changxiao Liu proposed a new thinking of about Q-markers in TCM and clarified that the five principles of Q-marker are uniqueness, validity, transmission and traceability, measurability, and matching environment. (Liu et al., 2016) The fingerprint of TCM can comprehensively and quantitatively analyze the chemical information contained in TCM, which is an important means of quality control of it. At the same time, it also becomes an important means in the research of substance standard of single-flavor drugs and compound formulas of TCM (Wei et al., 2018). The complexity of the TCM components determines that it is challenging to express the quality by single ingredient or index evaluation, so the model of multi-component quality control is proposed. QAMS is a multi-index quality evaluation model that is suitable for difficult preparation, high cost or unstable reference product by selecting a stable and easily prepared component as the internal reference within the corresponding linear range through the internal functional relationship between components in the sample to be tested (Wang et al., 2006). The two methods have been widely used in the quality control research of TCM herbs, pieces, and compounds. Based on the theory of systems biology, network pharmacology explores the correlation between drugs and diseases from a holistic perspective by establishing prediction models of the relationship between drugs and targets, and between diseases, and therapeutic targets (Wang et al., 2021). It examines the potential functional ingredients and targets of Chinese medicine compounds at a systematic level, and also theoretically elucidates the effectiveness of herbal medicines. At present, the studies of WYZW include fingerprint similarity evaluation, network pharmacology screening of potential targets and pathways, and QAMS (Liao et al., 2021a, 2021b, Wang et al., 2022a, 2022b, Tong et al., 2023), but few of them analyze Q-markers in combination with fingerprint, network pharmacology and QAMS. Therefore, based on Q-Marker theory, this study established an analysis method combining fingerprint, network pharmacology and QAMS, analyzed its components from the substance component level, predicted its potential targets and pathways from the molecular level, and finally predicted the Q-marker of WZYZW. The purpose of this study was to provide ideas for the new research model of WZYZW, to provide a basis for the quality control of WZYZW, and to provide a reference for the study of the mechanism of WZYZW.

2. Materials and methods

2.1. Materials

Shimadzu-ZU LC-2030C 3D high performance liquid chromatograph (Shimadzu Company, Japan), RC30002 electronic balance (Chengdu Bessec Instrument Research Institute), SQP 1/100,000 analytical balance (Sartorius company, Germany), PS-80A ultrasonic cleaner (Dongguan Jiekang Ultrasonic Equipment Co. Ltd.), etc.

The reference substances gallic acid (Batch no. 110831–201605), chlorogenic acid (Batch no. 110753–202018), ellagic acid (Batch no. 111959–201602), hyperoside (Batch no.111521–201205), rutin (Batch no.100080–201409), Schisandrol A (Batch no.110857–201815) were acquired from National Institutes for Food and Drug Control (Beijing China); Geniposidic acid (Lot no. CHB190117), Verbascoside(Lot no. CHB171103), Astragalin (Lot no.CHB201224), kaempferol (Lot no. CHB170224), Schisandrol B (Lot no.CHB190212), Schisandrin B (Lot no. CHB1800126) were obtained from Chroma Biotechnology Co. Ltd. (Chengdu China). Acetonitrile and phosphoric acid are chromatographically pure, and the rest of the reagents are analytical pure. 10 batches of WZYZW for experiments, specifications: 60 g/bottle, Lot no.s are 19035048, 19035103, 20035275, 20035279, 21035111, 21035114, 21035125, 21035362, 21035362, 22035102, 22035108, which were acquired from Beijing Tongrentang Co., Ltd. (Beijing China).

2.2. Chromatographic conditions

The column was InerSustainTM C18 (250 mm × 4.6 mm, 5 um); Mobile phase: acetonitrile (A)-0.4 % phosphoric acid solution (B), gradient elution: (0 ~ 5 min, 5 %A, 5 ~ 10 min, 5 %~7%A; 10 ~ 15 min, 7 %~9%A; 15 ~ 20 min, 9 %~10 %A; 20 ~ 25 min, 10 %~16 %A; 25 ~ 35 min, 16 %A; 35 ~ 40 min, 16 %-17 %A; 40 ~ 50 min, 17 %~18 %A; 50 ~ 75 min, 18 %~60 %A; 75 ~ 85 min, 60 %~65 %A; 85 ~ 95 min, 65 %~70 %A), Wavelength of detection: 254 nm; Temperature of column:40 °C; Flow rate:1.0 mL/min; Volume of injection:10 μL.

2.3. Preparation of standard solutions

Precision weighing gallic acid, geniposidic acid, hyperoside, chlorogenic acid, rutin, ellagic acid, astragalin, verbascoside, kaempferol, schisandrol A, schisandrol B, schisandrin B reference substance, add methanol to dissolve, to make a concentration of 216, 999, 392, 404, 622, 504, 412, 300, 228, 512, 504, 848 μg/mL of the standard solution, take an appropriate amount of each standard solution, then diluting with methanol, a mixed reference solution with concentrations of 8.64, 39.984, 7.84, 52.52, 24.88, 34.272, 10.712, 22.2, 5.928,

11.264, 10.08 and 15.264 μg/mL was obtained and stored at 4 °C.

2.4. Preparation of test solutions

Take an appropriate amount of WZYZW, crush (through a 60 mesh sieve), take about 2 g of powder, weigh it accurately, put it in a stopper Erlenmeyer flask, add 70 % methanol 50 mL, weigh it, ultrasonic (500 W, 40 kHz) for 60 min, cool, weigh. The lost weight was made up of 70 % methanol, shaken well, and filtered through a 0.45 μm microporous membrane, and the filtrate was obtained.

2.5. Network pharmacology analysis

The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) is used to search for SMILES of composition. The target of the components was predicted using the Swiss Target Prediction Database (https://swisstargetprediction.ch/). The protein–protein interaction (PPI) analysis of the target was visualized using the string database (https://cn.string-db.org/) and Cytoscape 3.7.2 software. GO and Kyoto Genes and Genomes Encyclopedia (KEGG) enrichment based on the overlapping targets of the major compounds identified from WZYZW were analyzed by DAVID database (https://david.ncifcrf.gov/). A comprehensive network of "Drug-components-targets-pathways" was built to explain the efficacy of the chemical ingredients.

2.6. Statistical analysis

The professional software "Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2012)" was used to acquire Similarity analysis (SA), which was recommended by the National Medical Products Administration of China.

3. Results

3.1. Establishment fingerprint of WZYZW

3.1.1. Method validation

3.1.1.1. Precision. Take WZYZW (S5) test solution, according to "1.2", inject 6 times continuously, and record the chromatogram. The similarity was greater than 0.90, the results showed that the instrument has good accuracy.

3.1.1.2. Repeatability test. Six test samples of WZYZW (S5) were prepared in parallel according to the method in "1.4", injected according to the chromatographic conditions in "1.2" and the chromatograms were recorded. The similarity was greater than 0.99 and the results exhibited that the method was reproducible.

3.1.1.3. Stability test. The test solution of WZYZW (S5) was injected into the HPLC at 0, 2, 4, 8, 10, and 24 h according to the conditions in "1.2", recording the chromatogram. The similarity was greater than 0.93, and the results showed that the test solution had a good stability in 24 h.

3.1.2. Fingerprint mapping and similarity valuation

Take ten lots of WZYZW samples to prepare test solutions according to "1.4", inject samples according to the chromatographic conditions under "1.2", and the data with AIA style was imported into the "Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine". The S1 sample was set as a reference spectrum (median method with a time width of 0.1) (Fig. 1). A total of 28 common peaks were calibrated, and we identified 12 characteristic peaks by comparing them with the reference standards (Fig. 2), of which peak 1 was gallic acid, peak 3 was geniposidic acid, peak 8 was chlorogenic acid, peak 16 was ellagic acid, peak 17 was rutin, peak 18 was hyperoside, peak 19 was verbascoside, peak 20 was astragalin, peak 23 was kaempferol, peak 25 was Schisandrol A, peak 26 was Schisandrol B, and peak 28 was schisandrin B. The structures of the 12 components are

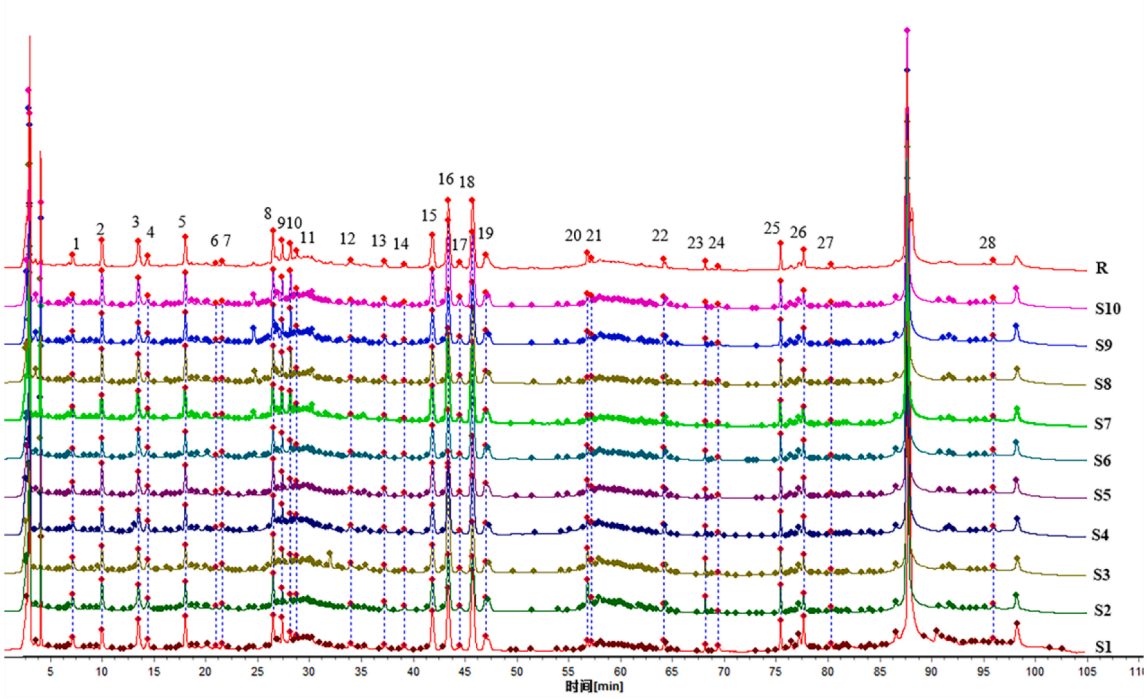


Fig. 1. HPLC fingerprints for ten lots (S1 ~ S10) of WZYZW, and batch R is the simulated chromatogram.

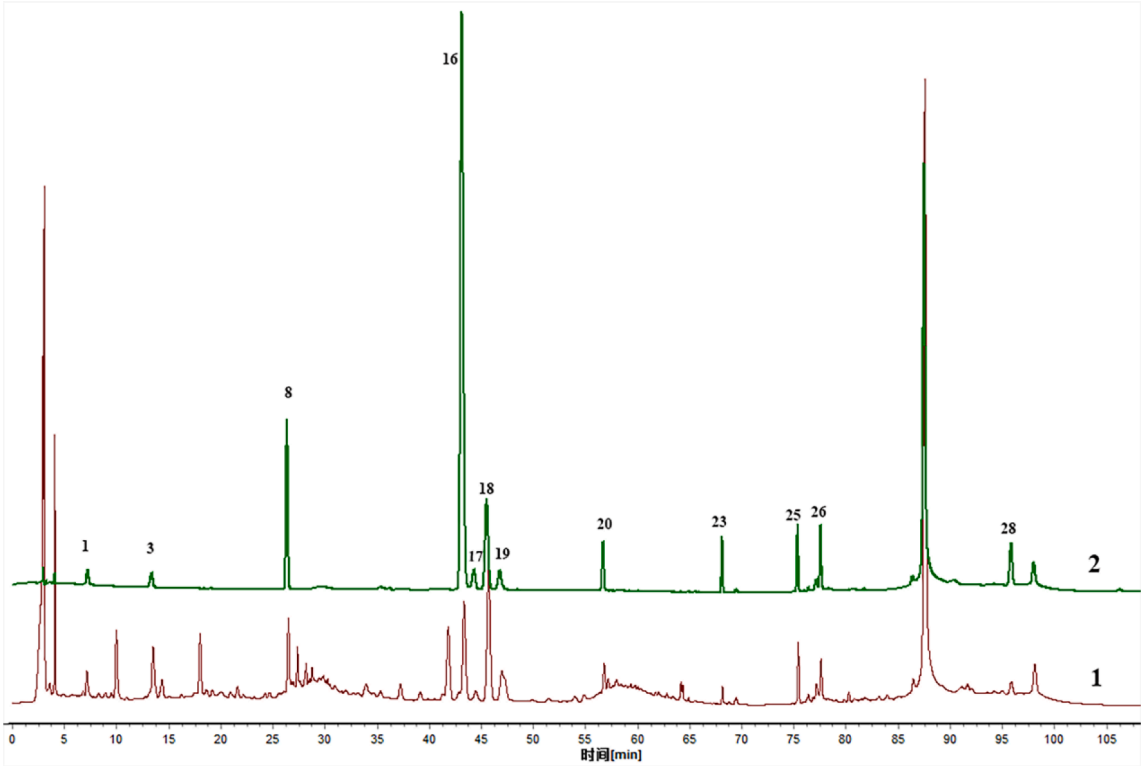


Fig. 2. HPLC chromatogram of WZYZW test solution (1) and mixed standard solution (2). Note. 1, gallic acid. 3, geniposidic acid. 8, chlorogenic acid. 16, ellagic acid. 17, rutin. 18, hyperoside. 19, verbascoside. 20, astragalin. 23, kaempferol. 25, schisandrol A. 26, schisandrol B. 28, schisandrin B.

shown in Fig. 3. In Table 1, we exhibited the SA information, in which the similarity of S2, S5, S6, and S8 is less than 0.9, indicating that there is a difference in quality between different batches of WZYZW, and the results of cluster analysis of each batch are shown in Fig. 4.

3.2. Network pharmacology predictive analysis

3.2.1. Prediction of compound targets

The SMILES of 12 compounds of gallic acid, geniposidic acid, hyperoside, rutin, chlorogenic acid, astragalin, ellagic acid, verbascoside, kaempferol, schisandrol A, schisandrol B, and schizandrin B were searched in the Pub Chem database, and SMILES was imported into the Swiss Target Prediction database for predictive analysis of the corresponding targets. Removal of repeated targets, we yielded 276 targets in all associated with 12 compounds.

3.2.2. PPI network construction

The 268 target proteins obtained were imported into the STRING database, set species selection as homo sapiens, and the highest confidence protein interaction parameter score value installed was >0.9, other parameter settings remain unchanged, remove a single node in the network, and obtain a PPI network diagram, see Fig. 5**-a.** The topological characterization of PPI is shown in Fig. 5**-b**, where the color was darker and the shape was larger, indicating that the target was more important. Points with a degree, betweenness, and closeness greater than the median and degree ≥ 35 were selected as core targets. 41 key targets in total were obtained after screening. The top 20 were AKT1, TNF, EGFR, SRC, HSP90AA1, STAT3, JUN, MTOR, ESR1, ERBB2, PTGS2, MMP9, PIK3CA, BCL2L1, MDM2, KDR, IL2, PIK3R1, JAK2 and AR **(**Table 2**)**.

3.2.3. GO enrichment and KEGG pathway analysis

GO (Gene Ontology) functional and KEGG pathway enrichment analysis of 41 potential core target were performed using the DAVID 2021 database. Both analysis had statistical significance ($P < 0.05$).

After GO enrichment analysis, some significant items (20 biological processes (BP), 15 molecular functions (MF), and 15 cellular components (CC)) were selected for display, as shown in Fig. 6. The results of GO analysis showed that BP was significantly gathered in protein phosphorylation、signal transduction, intracellular, signal transduction, positive regulation of gene expression and negative regulation of the apoptotic process. MF is mainly involved in the regulation of protein, adenosine triphosphate (ATP), the activity of protein kinase, transmembrane receptor protein tyrosine kinase, protein kinase, etc. CC is mainly enriched in cell fluid, cytoplasm, plasma membrane, etc.

140 pathways in total were gained by the KEGG enrichment study, and the pathway with a related gene number greater than 20 was chosen for display, as shown in Fig. 7, the enriched pathways mainly involved pathways in cancer, PI3K-Akt signaling pathway, proteoglycans in cancer, Endocrine resistance, EGFR tyrosine kinase inhibitor resistance, prostate cancer, Fluid shear stress and atherosclerosis, which indicated that these 41 core targets may be mainly regulated by regulating these pathways to achieve the purpose of intervening in disease.

3.2.4. "Drug-components-targets-pathways" network construction

The "drug-components-targets-pathways" network was constructed using Cytoscape 3.7.2 software based on the screened 12 components, 41 targets, and 20 pathways, and the results are shown in Fig. 8. The figure showed that WZYZW achieves its therapeutic effect through the synergistic effect of various components, various targets, and various pathways, and there was a close connection between ingredients, targets, and pathways; Among the 41 core targets, the connectivity of PIK3CA, AKT1, PIK3CB, PIK3CD, PIK3R1, EGFR, SRC, GSK3B, MAP2K1, PTK2, and IGF1R was higher than other targets, which may be the key target for WZYZW to exert efficacy. Among the 20 pathways, there is little difference in connectivity and all are likely to be key signaling pathways for WZYZW.

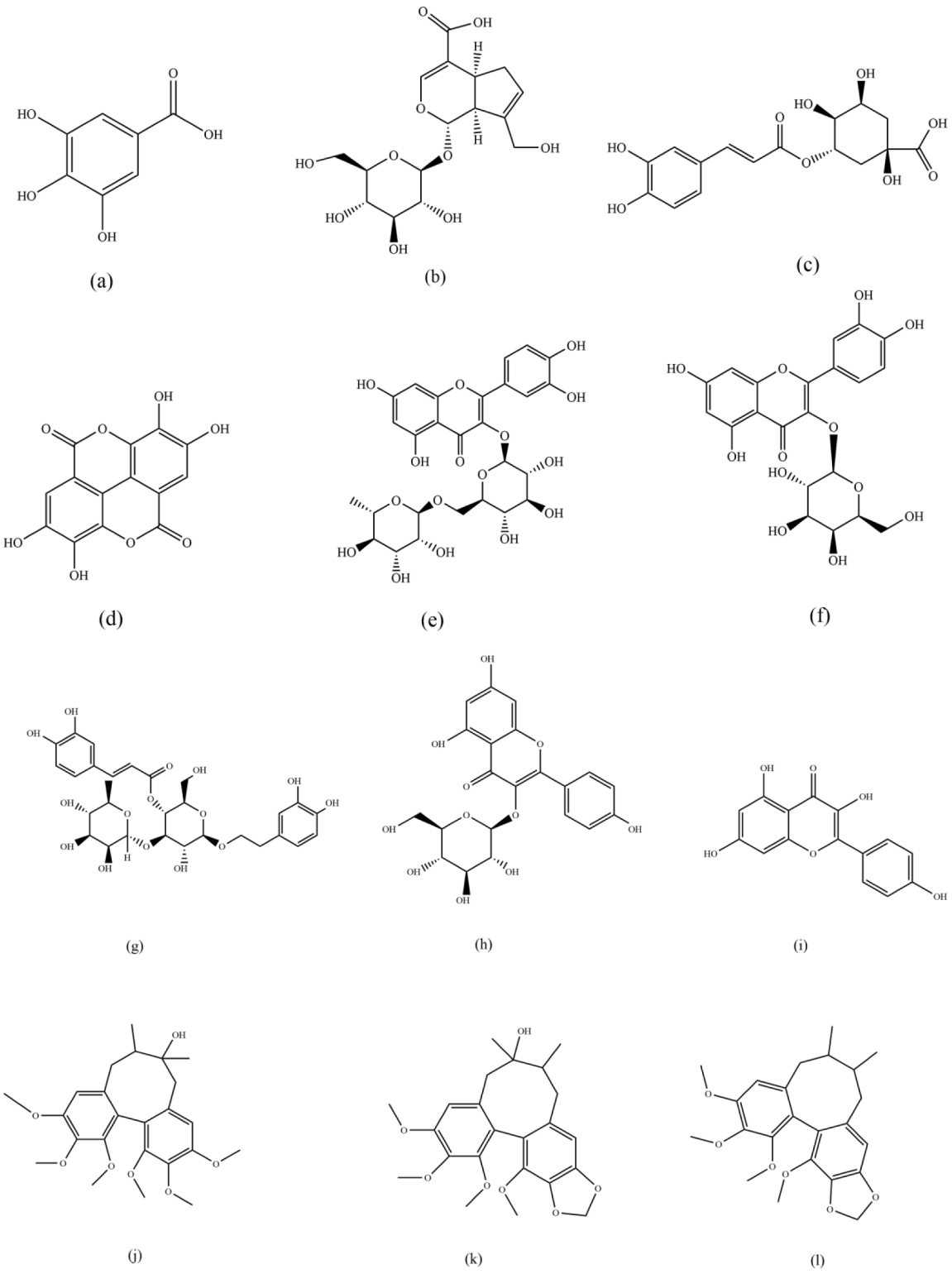


Fig. 3. Chemical structures of gallic acid (a), geniposidic acid (b), chlorogenic acid (c), ellagic acid (d), rutin (e), hyperoside (f), verbascoside (g), astragalin (h), kaempferol (i), schisandrol A (j), schisandrol B (k), schisandrin B (l).

3.2.5. Integrated analysis

As a classic prescription for tonifying the kidney and invigorating essence, WZYZW is comprehensively used in clinical applications with significant efficacy in the treatment of reproductive, tumor, urinary, nerve, and other diseases (Duan et al., 2015, Fu et al., 2017). GO enrichment analysis showed that WZYZW had a wide range of regulatory effects on cell signal transduction, positive regulation of gene expression, negative regulation of the apoptosis process, the activity of protein kinase and transmembrane receptor protein tyrosine kinase. In the KEGG enrichment analysis, WZYZW mainly regulates the activities of PI3K-Akt signaling and cancer-related receptor activity.

Schisandrol A is one of the main component of WZYZW, which can exert neuroprotective effects via stimulating the PI3K/Akt pathway, inhibiting the IKK/nuclear factor κB inhibitory protein pathway,

Table 1
SA results for 10 batches of WZYZW samples.

No.	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	R
S1	1.000	0.965	0.896	0.963	0.983	0.991	0.979	0.983	0.983	0.970	0.986
S2	0.965	1.000	0.936	0.983	0.984	0.964	0.966	0.962	0.984	0.974	0.986
S3	0.896	0.936	1.000	0.971	0.889	0.876	0.952	0.892	0.920	0.961	0.946
S4	0.963	0.983	0.971	1.000	0.964	0.958	0.985	0.962	0.981	0.990	0.991
S5	0.983	0.984	0.889	0.964	1.000	0.982	0.965	0.981	0.989	0.964	0.984
S6	0.991	0.964	0.876	0.958	0.982	1.000	0.973	0.990	0.984	0.967	0.982
S7	0.979	0.966	0.952	0.985	0.965	0.973	1.000	0.978	0.981	0.995	0.993
S8	0.983	0.962	0.892	0.962	0.981	0.990	0.978	1.000	0.991	0.980	0.986
S9	0.983	0.984	0.920	0.981	0.989	0.984	0.981	0.991	1.000	0.986	0.995
S10	0.970	0.974	0.961	0.990	0.964	0.967	0.995	0.980	0.986	1.000	0.995
R	0.986	0.986	0.946	0.991	0.984	0.982	0.993	0.986	0.995	0.995	1.000

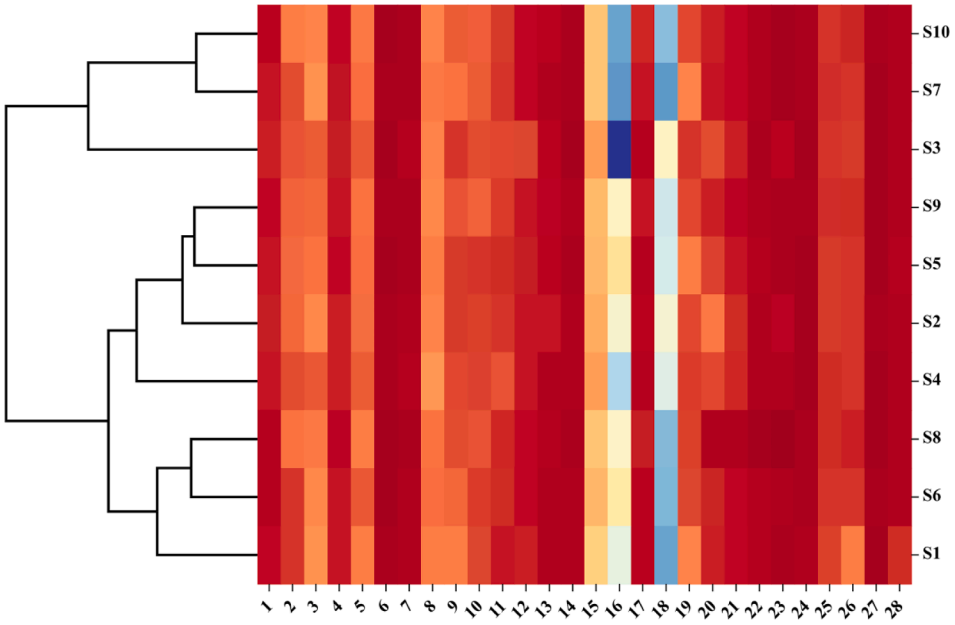
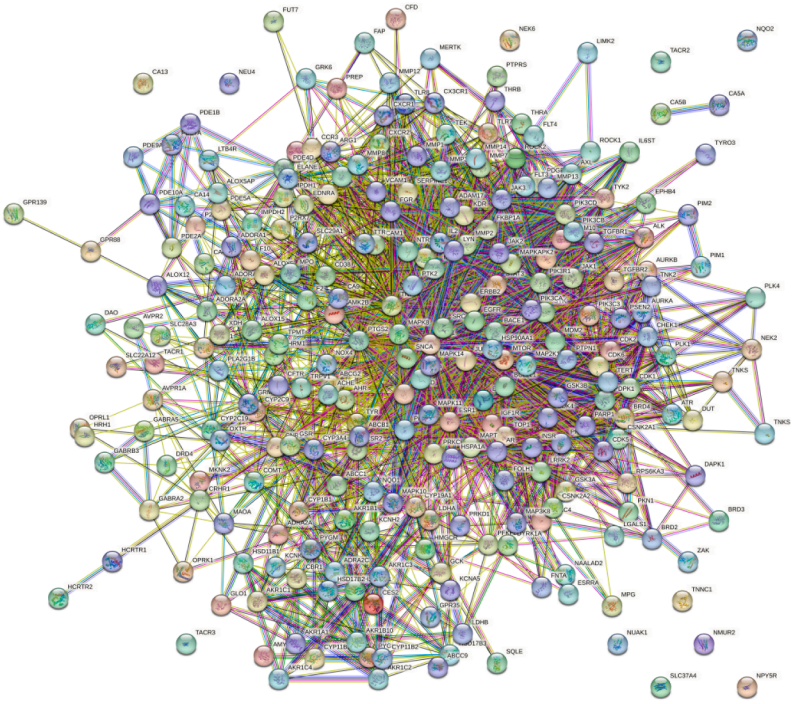


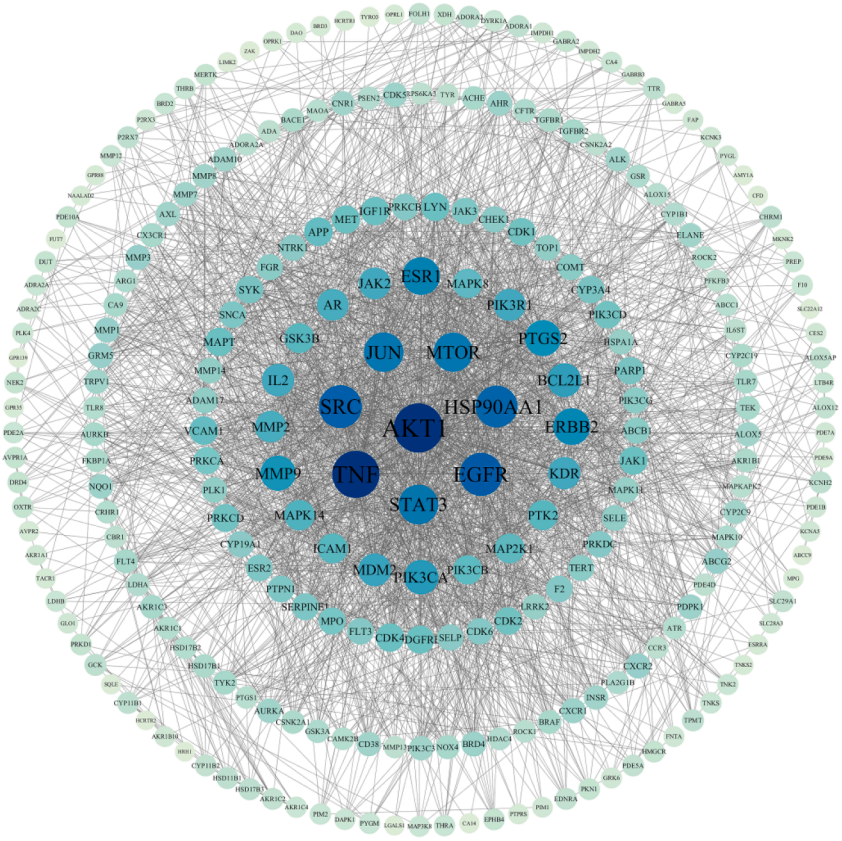
Fig. 4. Clustering analysis of 10 batches of WZYZW.

reducing neuronal inflammation and oxidative stress response (Yan et al., 2019), and has protective effects on depression and cerebral ischemia (Ru et al., 2022); Ellagic acid could protective against cerebral ischaemia and kidney injury (Chen et al., 2021a, 2021b, 2021c Sun et al., 2022); Kaempferol can regulate the phosphorylation level of Akt protein in brain tissue to exert brain protective effects (Xu et al., 2022a, 2022b), can also restrain the multiplication of human colorectal cancer cells and induce their apoptosis, and downregulate the PI3K/Akt signaling pathway to induce autocannibalism in cancer cells (Gu et al., 2020); Researches have diaplayed that the pharmacological effects of hyperoside are manifested in the protection, anti-tumor, antidepressant and other aspects of kidney and liver (Chen et al., 2019, Liang et al., 2020, Zhu et al., 2022), AKT1 can be activated by extracellular signaling through PI3K-dependent mechanisms, is the core factor in the PI3K/AKT signaling pathway, and is related to the expression of cancer (Herberts et al., 2020, Li et al., 2022), and phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) gene is closely related to the clinicopathological characteristics of breast cancer (Lin and Chen, 2022). The PI3K-AKT signaling pathway has also been associated with the treatment of depression, moreover the studies have shown that depression affects reproductive function, so WZYZW may modulate this signaling pathway to treat infertility (Huang et al., 2020, Zhu et al., 2021).

Bladder cancer is a common disease of the urinary system, studies have found that the process of bladder cancer will induce the expression of phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta (PIK3CB), p-AKT proteins, while PI3K/AKT signaling pathway is further activated, the connection of growth factors to their receptor tyrosine kinase (RTK) or G protein-coupled receptor (GPCR) stimulates the production of phosphatidylinositide 3-kinases (PI3K) isoform phosphatidylinositol, 3, 4, 5 triphosphate (PIP3), PIP3 as a second messenger, in turn, helps activate Akt, once activated, Akt can participate in a series of processes such as apoptosis and protein synthesis through phosphorylation pathways (Yang et al., 2021), and the application of WZYZW in urology may be related to inhibiting the activation of this signaling pathway. Epidermal growth factor receptor (EGFR) is a tyrosine kinase, the mutations in the EGFR gene and overexpression of proteins can cause cancer, and WZYZW's treatment for cancer may be to inhibit the activity of this enzyme, inhibit its gene mutation, and overexpression (Floc'h et al., 2020). WZYZW modulates the PI3K/Akt signaling pathway, phosphorylates Akt, activates the downstream transcription factor Nrf2, and thus playing a preventive role in the prevention and treatment of neural tube malformations through antioxidant stress and anti-apoptosis pathway (Chen et al., 2022a, 2022b), the above research shows that the results of network pharmacology prediction are close to the existing relevant literature, indicating that the research method has certain accuracy and reliability, and is also in line with the concept of the integrity of TCM. It provides a reference for further elaboration on the material basis of WZYZW. Multiple key components in WZYZW can act on multiple core targets, thereby intervening in multiple pathways and playing a role in treating or intervening in diseases. The 12 components identified in WZYZW have certain pharmacological effects, and



a



b

Fig. 5. PPI network.

Table 2
Key node information in the PPI network.

Target	Name	Degree	BetweennessCentrality
AKT1	Serine/threonine-protein kinase AKT	126	0.1412
TNF	TNF-alpha	118	0.1102
EGFR	Epidermal growth factor receptor erbB1	100	0.0490
SRC	Epidermal growth factor receptor erbB1	99	0.0709
HSP90AA1	Heat shock protein HSP 90-alpha	95	0.0524
STAT3	Signal transducer and activator of transcription 3	86	0.0222
JUN	Proto-oncogene c-JUN	85	0.0238
MTOR	Serine/threonine-protein kinase mTOR	84	0.0359
ESR1	Estrogen receptor alpha	78	0.0435
ERBB2	Receptor protein-tyrosine kinase erbB-2	74	0.0179
PTGS2	Cyclooxygenase-2	71	0.0322
MMP9	Matrix metalloproteinase 9	67	0.0132
PIK3CA	PI3-kinase p110-alpha subunit	64	0.0093
BCL2L1	Apoptosis regulator Bcl-X	61	0.0111
MDM2	p53-binding protein Mdm-2	57	0.0119
KDR	Vascular endothelial growth factor receptor 2	55	0.0060
IL2	Interleukin-2	55	0.0090
PIK3R1	PI3-kinase p85-alpha subunit	54	0.0070
JAK2	Tyrosine-protein kinase JAK2	53	0.0057
AR	Androgen Receptor	52	0.0267

the targets and pathways predicted by these components are relevant to the diseases treated by WZYZW. Combined with the essential request of Q-Markers, it can be preliminarily predicted that the 12 components are potential Q-markers for WZYZW. In order to further verify the rationality of this prediction, we will use QAMS to determine the content of these components in multiple batches of WZYZW.

3.3. Study on the quality control of WZYZW based on QAMS

3.3.1. Validation of methods

3.3.1.1. Linearity ranges. The linearity was determined under the chromatographic conditions of "1.3" by taking 2, 5, 10, 15, 20, and 25 μL of the mixed standards. The results showed that the 12 components showed good linearity in the range of determination (Table 3).

3.3.1.2. Precision. Take 10 μL of the mixed standard solution under "1.3", inject 6 times according to the chromatographic conditions under "1.2", record the chromatography peak area of 12 components, and calculate the relative standard deviation (RSD). For 3 consecutive days, 3 injections were analyzed 3 times a day, recording the peak area of each component, and calculating the RSD value as the daytime precision. The results showed that the intraday and interday precision RSD were lower than 3.0 %, which showed that the instrument has a well precision.

3.3.1.3. Test of stability. About 2 g of WZYZW (Lot no. 20035275) was taken, weighed precisely, and prepared according to the method under "1.4", and 10 μL of the sample was taken after 0, 2, 4, 6, 8, 12, and 24 h for the determination. The peak area of each component was recorded, and the RSD of them were computed as 1.77 %, 2.71 %, 1.94 %, 1.95 %,

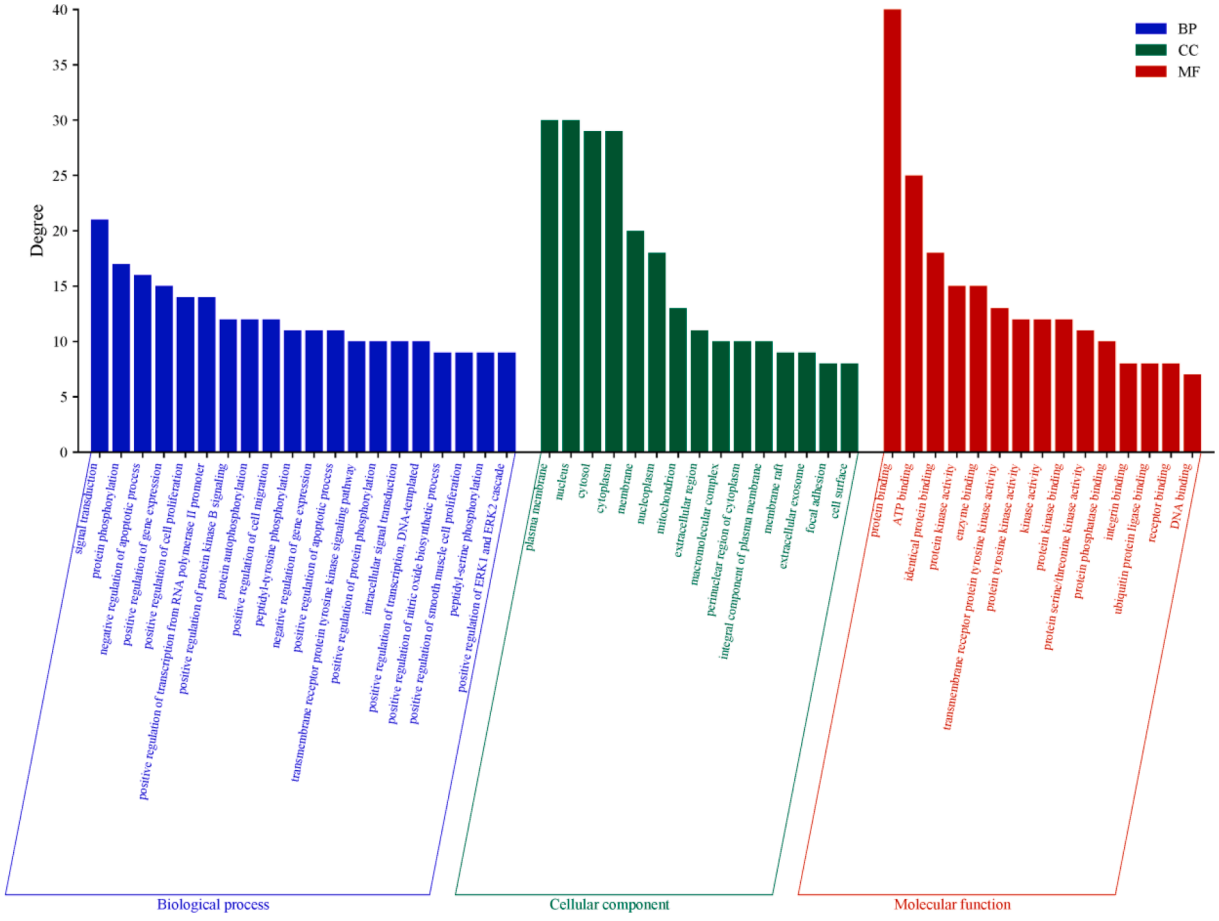


Fig. 6. GO enrichment analysis.

Fig. 7. KEGG enrichment analysis.

1.97 %, 0.95 %, 2.25 %, 2.59 %, 1.97 %, 0.96 %, 2.39 % and 1.36 %, which indicated that the test solutions were stable within 24 h. The stability of the test solution was good.

3.3.1.4. Repeatability test. About 2 g of WZYZW (Lot no. 20035275) was taken, weighed precisely, and prepared in parallel according to the method under "1.4", and six solutions were prepared in parallel. The average contents of the above 12 components were 0.1430, 1.7113, 0.5782, 0.1712, 0.1034, 0.9687, 1.1923, 0.1442, 0.0361, 0.2593, 0.3921 and 0.1042 mg/g, with RSD of 2.60 %, 0.82 %, 2.14 %, 2.67 %, 1.90 %, 0.76 %, 1.75 %, 2.16 %, 2.43 %, 2.49 %, 2.49 %, and 2.54 %, indicated that the method repeatability was good.

3.3.1.5. Recovery test. Accurately weigh 6 samples of WZYZW (Lot no. 20035275) with known content, 1.0 g respectively, place them into a 50 mL measuring flask, add an appropriate amount of mixed standard solution, prepare the test solution using the method described in "1.4", and inject into HPLC using the chromatographic conditions described in "1.2", and calculated the average recovery rates of the above 12 components to be 97.93 %, 101.185 %, 99.25 %, 100.35 %, 98.73 %, 98.67 %, respectively. 102.96 %, 100.78 %, 99.90 %, 98.32 %, 98.06 %, 100.24 %, RSD values were 2.12 %, 2.29 %, 2.98 %, 2.61 %, 3.05 %, 2.24 %, 2.74 %, 2.58 %, 2.54 %, 2.98 %, 2.80 % and 3.02 %. The results demonstrated that the proposed method was accurate.

3.3.2. QAMS method establishment

3.3.2.1. Calculation of relative Correction factors (RCF). The reference solution was taken under "1.3", injected 3, 5, 10, 15, and 25 μL respectively, and the peak area of 12 components was determined. According to the formula $f_{s/k} = (W_k A_s)/(W_s A_k)$ (Wang et al., 2011), where A_s is the peak area of the internal parameter, W_s is the mass concentration of the internal component, A_k is the peak area of the target component k, and W_k is the mass concentration of the target component k. Hyperoside (H) was used asthe internal reference, and the $f_{s/k}$ between gallic acid (G1), geniposidic acid (G2), chlorogenic acid (C), ellagic acid (E), rutin (R), verbascoside (V), astragalin (A), kaempferol (I), Schisandrol A (S), Schisandrol B (D), Schisandrin B (K) were calculated respectively, and the results were shown in Table 4.

3.3.2.2. Reproducibility investigation of RCF. Take the mixed standards solution under "1.3", inject it according to the chromatographic conditions under "1.2", and investigate different liquid phase systems (Shimadzu-ZU LC-2030C 3D, Thermo ulti Mate 3000), different columns

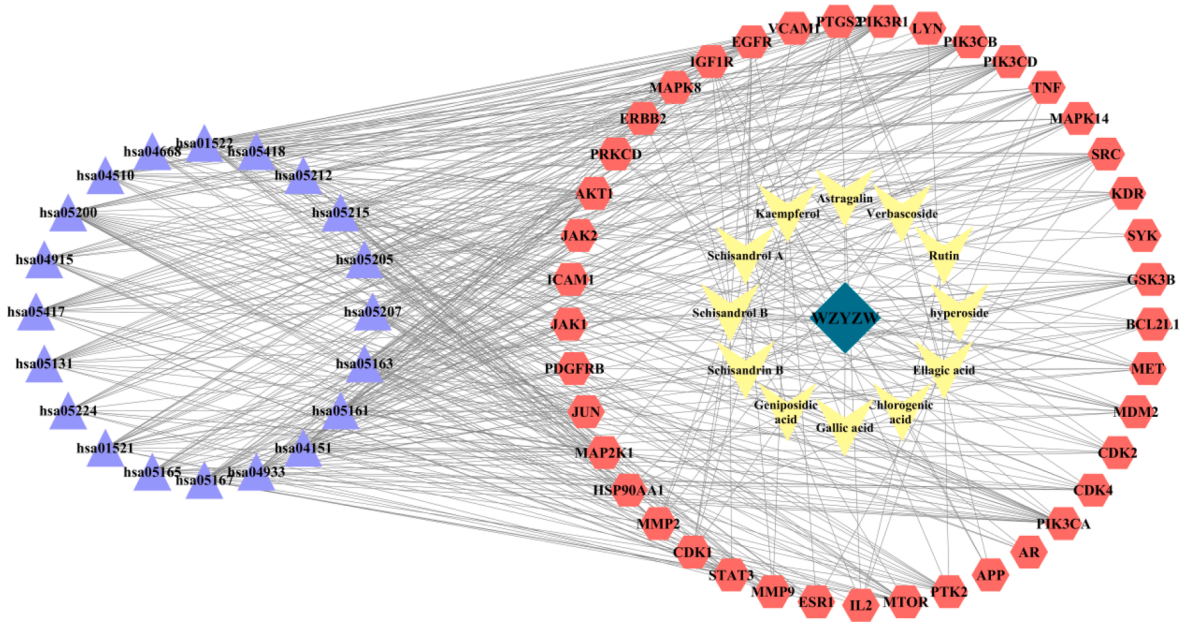


Fig. 8. Network of "drug-components-targets-pathways". Blue represents pathways; Red represents targets; Yellow represents components; Green represents WZYZW. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Results of the linearity of the 12 ingredients in WZYZW.

Component	Linear equations	R^2	Linear range (μg)
Gallic acid	y = 1521451.47x + 7933.79	0.9991	0.017 ~ 0.216
Geniposidic acid	y = 409175x + 65913	0.9993	0.080 ~ 1.000
Chlorogenic acid	y = 1151940.41x + 28071.15	0.9992	0.105 ~ 1.313
Ellagic acid	y = 10446176.75x + 32414.76	0.9998	0.069 ~ 0.857
Rutin	y = 1776086.60x + 1049.17	0.9998	0.016 ~ 0.196
Hyperoside	y = 2739861.07x-3247.99	0.9996	0.045 ~ 0.512
Verbascoside	y = 656053x-5264.7	0.9994	0.044 ~ 0.555
Astragalin	y = 1768619.22x + 2284.74	0.9997	0.021 ~ 0.268
Kaempferol	y = 2429590.84x-283.72	0.9995	0.012 ~ 0.148
Schisandrol A	y = 1721860.20x + 2387.57	0.9998	0.023 ~ 0.282
Schisandrol B	y = 1342199.59x + 81836.56	0.9992	0.020 ~ 0.252
Schisandrin B	y = 1506818.25x + 411.89	0.9995	0.031 ~ 0.382

(InerSustainTM C18, Kromasil 100-5-C18, all specifications (250 mm × 4.6 mm, 5 μm) and 2 different laboratories. The results showed that the RSD of the RCF of the 11 components was lower than 5 % (Table 5), revealing that there was no significant difference in the RCF of each component in WZYZW by different HPLC systems, different columns, and different laboratories. It showed that this method has a good durability.

3.3.2.3. Position of chromatographic peak. The chromatographic peaks to be measured can be more accurately located using the relative retention method. The formula is $r_{i/s} = t_{R(s)}/t_{R(i)}$ (Xue et al., 2023), the "i" stands for the component to be measured and the "s" for the reference component.The method under "1.2" was used for measuring the relative retention time of other component peaks and reference peaks with hyperoside as the reference peak, and the reproducibility of the relative retention time of different columns and different brands of instruments was investigated, the RSD of the relative retention time of

Table 4
$f_{s/k}$ of each component reference substance (n = 6).

Injection volume (μL)	$f_{H/G1}$	$f_{H/G2}$	$f_{H/C}$	$f_{H/E}$	$f_{H/R}$	$f_{H/V}$	$f_{H/A}$	$f_{H/I}$	$f_{H/S}$	$f_{H/D}$	$f_{H/K}$
3	1.6948	5.5750	2.1527	0.2560	1.5012	4.3813	1.4802	1.0883	1.4873	1.3403	1.7154
5	1.6481	5.6787	2.1313	0.2573	1.5193	4.3481	1.4961	1.1072	1.4750	1.3759	1.7039
10	1.6456	5.5817	2.2255	0.2583	1.4495	4.3337	1.5457	1.1504	1.4430	1.3596	1.7093
15	1.6936	5.6012	2.1436	0.2488	1.5048	4.3235	1.5152	1.1762	1.4908	1.3697	1.7187
20	1.6925	5.5708	2.1870	0.2636	1.5091	4.3436	1.5341	1.1278	1.4852	1.3323	1.7102
25	1.6608	5.5499	2.1877	0.2533	1.4990	4.3395	1.4976	1.1490	1.5068	1.2340	1.7177
Mean	1.6726	5.5929	2.1713	0.2562	1.4972	4.3449	1.5115	1.1331	1.4813	1.3353	1.7125
RSD	1.41 %	0.81 %	1.62 %	1.94 %	1.63 %	0.45 %	1.65 %	2.82 %	1.45 %	3.92 %	0.33 %

Table 5
Durability of RCF.

Lab.	Chromatographic Column		$f_{H/G1}$	$f_{H/G2}$	$f_{H/C}$	$f_{H/E}$	$f_{H/R}$	$f_{H/V}$	$f_{H/A}$	$f_{H/I}$	$f_{H/S}$	$f_{H/D}$	$f_{H/K}$
Lab.1	Shimadzu-ZU LC-2030C	InerSustain^{TM} C_{18}	1.6726	5.5929	2.1713	0.2562	1.4972	4.3449	1.5115	1.1331	1.4813	1.3353	1.7125
	3D	Kromasil 100–5-C_{18}	1.6480	5.5651	2.1286	0.2651	1.5328	4.4944	1.5388	1.1217	1.4491	1.3974	1.7886
Lab.2	Thermo Ulti Mate U3000	InerSustain^{TM} C_{18}	1.6121	5.5598	2.1932	0.2523	1.4945	4.3683	1.6029	1.1280	1.5105	1.3560	1.8082
		Kromasil 100–5-C_{18}	1.6106	5.5694	2.1843	0.2513	1.5342	4.3069	1.5712	1.1529	1.4247	1.3431	1.8029
Mean			1.6358	5.5718	2.1693	0.2562	1.5147	4.3786	1.5561	1.1339	1.4664	1.3579	1.7781
RSD%			1.83 %	0.26 %	1.32 %	2.46 %	1.44 %	1.86 %	2.55 %	1.19 %	2.55 %	2.04 %	2.50 %

each component was less than 5 % (Table 6), indicating that the relative retention time could be used to locate each component.

3.3.2.4. Determination of $f_{s/k}$. According to the above results of the system durability and repeatability test of $f_{s/k}$, the average value of the measurement results was taken to determine that the $f_{s/k}$ of hyperoside for geniposidic acid, chlorogenic acid, ellagic acid, rutin, verbascoside, astragalin, kaempferol, Schisandrol A, Schisandrol B, Schisandrin B were finally determined as 1.6358, 5.5718, 2.1693, 0.2562, 1.5147, 4.3786, 1.5561, 1.1339,1.4664, 1.3579 and 1.7781.

3.3.2.5. QAMS vs. ESM results comparison. Different batches of WZYZW, numbered 1 ~ 10, were collected, and the test solution was prepared according to the method under "1.4", and the prepared test solution and the mixed reference solution were determined by 10 μL according to the chromatographic conditions under the item of "1.2". The contents of 12 components were calculated by QAMS and ESM respectively, and the results were shown in Table 7. The calculation formula of percentage difference (PD) is as follows. PD = (QAMS − ESM) /[(QAMS + EMS)/2] × 100 %(Zhang et al., 2017). We can find that the PD of the two methods was less than ± 5.0 %, that was there were no significant differences between QAMS and ESM, which illustrated that the established method was accurate and reliable.

4. Discussion

The quality of TCM is the lifeline of it and related industries, which has a bearing on clinical efficacy and the quality development of the TCM industry. The establishment of Q-Markers for TCM can promote quality control research and strengthen the quality evaluation system for further improvement.

In this research, the selection of the extraction process, mobile phase composition, flow rate, column temperature, detection wavelength, and chromatographic conditions of the test solution was based on the "2020" version of Chinese Pharmacopoeia (Ch.P.) (Chinese Pharmacopoeia Commission, 2020), combined with the preliminary research (Liao et al., 2021a, 2021b), and finally determined that 0.4 % phosphate-acetonitrile was used as the mobile phase, the volume flow was set to 1.0 mL/min, set the column temperature as 40℃, and set the wavelength as 245 nm so that the reference and test products had a suitable degree of resolution under such conditions. Therefore, based on these conditions, 10 batches of WZYZW fingerprints were established, and 12 components were identified from 28 common peaks.

In order to further search for the potential targets and pathways of these components, we used network pharmacology to analyze it from the perspective of effectiveness. The results showed that the 12 active components acted on cancer pathways, PI3K-Akt signaling pathways, prostate cancer, and other key targets by regulating PIK3CA, AKT1, PIK3CB, PIK3R1, EGFR, SRC, GSK3B, MAP2K1, PTK2, IGF1R to play the therapeutic role. Numerous investigations have shown that the 12 components have significant pharmacological effects on neurological diseases, liver damage, kidney damage, anticancer, anti-tumor, anti-testicular inflammation, etc. by multiple targets and pathways. As a result, we used the QAMS approach to assess the quality of WZYZW based on the 12 components.

Modern pharmacological research have revealed that these 12 components have considerable effects, and the interaction of these pharmacological effects is associated with the therapy of WZYZW. Geniposidic acid can increase the expression of growth-associated protein 43(GAP43) through activation PI3K/AKT pathway. Ultimately, it improved nerve damage in mice with Alzheimer's disease (AD), thereby alleviating AD (Chen et al., 2022a, 2022b); as a novel NLRP3-specific covalent inhibitor, geniposidic acid protects against liver injury due to excessive accumulation of bile acids by inhibiting its expression, thereby reducing bile acid-induced inflammation, and by participating in the

Table 6
The relative retention values on various instruments and chromatographic columns.

Chromatographic Column		Relative retention time										
		Gallic acid	Geniposidic acid	Chlorogenic acid	Ellagic acid	Rutin	Verbascoside	Astragalin	Kaempferol	Schisandrol A	Schisandrol A	Schisandrol A
Shimadzu-ZU LC-2030C 3D	InerSustain™ C_{18}	6.1985	3.3594	1.7232	1.0530	1.0265	0.9510	0.8049	0.6007	0.5656	0.5572	0.4536
	Kromasil 100–5-C_{18}	6.8373	3.3097	1.6804	1.0593	1.0261	0.8789	0.7811	0.5649	0.5257	0.5122	0.4167
Thermo Ulti Mate U3000	InerSustain™ C_{18}	6.6596	3.6389	1.7212	1.0536	1.0324	0.9512	0.7895	0.5683	0.5660	0.5591	0.4544
	Kromasil 100–5-C_{18}	6.8493	3.6214	1.7176	1.0510	1.0267	0.8859	0.7890	0.5373	0.5205	0.5192	0.4191
Mean		6.7821	3.5234	1.7064	1.0546	1.0284	0.9053	0.7865	0.5568	0.5374	0.5302	0.4301
RSD%		4.59 %	4.94 %	1.19 %	0.34 %	0.29 %	4.34 %	1.26 %	4.57 %	4.54 %	4.60 %	4.78 %

Table 7
Contents of 12 components in WZYZW by QAMS and ESM (%,n = 3).

Component	Method	1	2	3	4	5	6	7	8	9	10
Hyperoside	ESM	0.0969	0.0678	0.0645	0.0735	0.0759	0.0947	0.1020	0.0944	0.0775	0.0927
Gallic acid	ESM	0.0148	0.0183	0.0185	0.0171	0.0160	0.0108	0.0164	0.0091	0.0146	0.0126
	QAMS	0.0146	0.0178	0.0180	0.0167	0.0157	0.0110	0.0160	0.0094	0.0144	0.0126
	PD/%	−1.3269	−2.6992	−2.7427	−2.2841	−1.8160	1.4631	−2.0876	3.3548	−1.1561	0.0087
Geniposidic acid	ESM	0.1710	0.1616	0.1272	0.1019	0.1361	0.1613	0.1629	0.1447	0.1193	0.1710
	QAMS	0.1744	0.1668	0.1381	0.1168	0.1454	0.1663	0.1677	0.1525	0.1314	0.1744
	PD/%	1.9858	3.1648	4.5858	4.3514	−0.4657	3.0807	2.8662	−1.4405	1.5977	0.0393
Chlorogenic acid	ESM	0.0579	0.0628	0.0618	0.0718	0.0639	0.0518	0.0579	0.0573	0.0641	0.0579
	QAMS	0.0582	0.0628	0.0619	0.0710	0.0637	0.0527	0.0582	0.0577	0.0640	0.0582
	PD/%	0.5541	−0.0237	0.1323	−1.1211	−0.2122	1.6132	0.5465	0.6621	−0.2525	−0.0008
Ellagic acid	ESM	0.0176	0.0166	0.0320	0.0214	0.0130	0.0140	0.0261	0.0162	0.0157	0.0176
	QAMS	0.0180	0.0170	0.0321	0.0217	0.0134	0.0145	0.0263	0.0166	0.0161	0.0180
	PD/%	2.0245	2.3986	0.3619	1.4101	3.5205	3.0412	0.6989	2.3674	2.5707	0.8129
Rutin	ESM	0.0106	0.0105	0.0096	0.0084	0.0098	0.0116	0.0150	0.0165	0.0157	0.0106
	QAMS	0.0106	0.0105	0.0097	0.0086	0.0095	0.0116	0.0149	0.0164	0.0156	0.0106
	PD/%	−0.2441	−0.0958	1.4003	2.7708	−3.6682	−0.3402	−0.6328	−0.6921	−0.5867	−0.7986
Verbascoside	ESM	0.1189	0.0758	0.0621	0.0667	0.1168	0.0744	0.1166	0.0684	0.0751	0.1192
	QAMS	0.1230	0.0778	0.0634	0.0682	0.1183	0.0714	0.1192	0.0712	0.0771	0.1182
	PD/%	3.4103	2.6134	2.0680	2.2331	1.2783	−4.1459	2.2459	4.0071	2.5395	−0.8174
Astragalin	ESM	0.0143	0.0419	0.0295	0.0259	0.0261	0.0198	0.0140	0.0127	0.0177	0.0143
	QAMS	0.0148	0.0426	0.0301	0.0264	0.0267	0.0203	0.0146	0.0122	0.0170	0.0148
	PD/%	2.8341	1.5855	1.9084	2.0004	1.9761	2.2708	3.8767	−4.2036	−3.7832	2.5009
Kaempferol	ESM	0.0037	0.0089	0.0086	0.0047	0.0042	0.0049	0.0021	0.0006	0.0034	0.0037
	QAMS	0.0037	0.0091	0.0087	0.0048	0.0043	0.0050	0.0022	0.0006	0.0034	0.0037
	PD/%	1.5861	1.2709	1.3051	1.5163	1.5695	1.4075	2.1193	−2.6276	1.7300	1.8311
Schisandrol A	ESM	0.0261	0.0242	0.0216	0.0212	0.0236	0.0225	0.0217	0.0214	0.0218	0.0221
	QAMS	0.0254	0.0237	0.0213	0.0220	0.0231	0.0221	0.0224	0.0211	0.0215	0.0227
	PD/%	−2.6266	−2.0985	−1.4176	3.2930	−2.0035	−1.7888	2.9571	−1.4855	−1.5441	2.8191
Schisandrol B	ESM	0.0400	0.0148	0.0153	0.0159	0.0166	0.0153	0.0148	0.0061	0.0114	0.0161
	QAMS	0.0393	0.0145	0.0159	0.0162	0.0167	0.0158	0.0153	0.0061	0.0112	0.0167
	PD/%	−1.7928	−2.0027	4.3009	2.0530	0.5741	3.1583	2.9218	−0.7932	−1.8248	3.5202
Schisandrin B	ESM	0.0103	0.0099	0.0084	0.0084	0.0102	0.0097	0.0092	0.0092	0.0092	0.0092
	QAMS	0.0102	0.0098	0.0084	0.0083	0.0101	0.0096	0.0090	0.0091	0.0091	0.0096
	PD/%	−1.3309	−1.1774	−1.0416	−1.0889	−1.2369	−1.2786	−1.2610	−1.2391	−1.1788	4.2892

regulation of bile transporters such as Bsep and Mrp2, thereby protecting against liver injury (Chen et al., 2016, Song et al., 2022), at the same time, geniposidic acid is also used in ischemic brain injury. Schisandrin B has shown strong pharmacological activity in anti-inflammatory (Chen et al., 2021a, 2021b, 2021c, Zhang et al., 2021), anti-tumor (Li et al., 2021a, 2021b), hepatoprotective (Wang et al., 2020, Chen et al., 2021a, 2021b, 2021c, Li et al., 2021a, 2021b, Yan et al., 2022), infertility (Zou et al., 2021) and other applications. Gallic acid can inhibit the production of colon cancer cells by regulating the formation of ferroptosis to play an anti-cancer effect (Hong et al., 2021), and other studies have shown that it exerts anti-tumor aversion by inhibiting the reproduction of cancer cells to make it apoptosis (Priyadarshi et al., 2021). A certain dose of gallic acid can also reduce the kidney tissue damage caused by diclofenac (DIC), Gallic acid treatment can significantly improve DIC-induced oxidative stress abnormalities and serum biochemical parameters (Moradi et al., 2021). AD is a persistent neurodegenerative disease manifested by neuroinflammation, mitochondrial dysfunction, increased oxidative stress, weakened antioxidant defenses, and elevated acetylcholinesterase activity. Other manifestation was amyloidosis in the brain, which was found that given gallic acid treatment to mice with the characteristics of the disease, amyloidosis β in the cerebral cortex and cerebral vessels was alleviated, and the mechanism was related to direct inhibition of β-site APP-cleaving enzyme 1 (BACE1) activity (Mori et al., 2020). As a dimer of gallic acid, ellagic acid protects neurons through antioxidant action, iron chelation, and mitochondrial protection (Javaid et al., 2021). Chlorogenic acid plays a pharmacological role in anti-inflammatory, neuroprotective, anti-cancer, etc. (Naveed et al., 2018, Munteanu and Apetrei, 2021). Hyperoside is a natural flavonoid with antitumor, anticancer, antidepressant, and other effects (Wang et al., 2022a, 2022b, Xu et al., 2022a, 2022b), which can inhibit the proliferation of hepatocellular carcinoma (HCC) by inhibiting the PI3K/AKT signaling pathway. The mechanism is that hyperoside can reduce the expression of bone morphogenetic protein 7 (BMP-7), thereby inducing cell cycle arrest in HepG2 cells (Wei et al., 2021). Verbascoside have pharmacological effects such as anticancer, neuropathy, and anti-testicular injury (Alipieva et al., 2014, Han et al., 2021, Wu et al., 2021). It is possible to improve sperm levels by resisting testicular damage, thus treating male infertility, which is consistent with the clinical application of WZYZW. Therefore, it is necessary to carry out a quality evaluation to provide a reference for the construction of the WZYZW quality evaluation system.

In summary, based on fingerprint and network pharmacology, this study preliminarily predicted that gallic acid, geniposidic acid, rutin, hyperoside, chlorogenic acid, ellagic acid, verbascoside, astragalin, kaempferol, schisandrol A, schisandrol B, and schisandrin B were the potential Q-markers of WZYZW, and conducted quality studies on 12 components in multiple batches of WZYZW, to provide a foundation and reference for further studies on quality control as well as theoretical support for in-depth studies on the mechanism of WZYZW. However, the current research on WZYZW is still insufficient. For example, the basis of some chemical substances related to traditional efficacy is not clear, and it still stays in the stage of crude extract researching, the research on pharmacological effects and mechanisms is not deep enough, and the quality control research is not systematic enough, so the development and utilization of WZYZW is limited to a certain extent. Therefore, further research on WZYZW can be conducted with the help of cutting-edge technologies such as metabolomics and mass spectrometry imaging according to the overall model of Q-Marker research.

5. Conclusion

In this study, a fingerprint of 10 batches of WZYZW was established. By comparing with the standards, 12 components were identified from 28 common peaks, which are gallic acid, geniposidic acid, rutin,

hyperoside, chlorogenic acid, astragalin, ellagic acid, verbascoside, kaempferol, schisandrol A, schisandrol B, and schisandrin B. Then, based on network pharmacology, the "drug-components-targets-pathways" network was constructed, its potential targets and pathways were analyzed. It was found that WZYZW can exert therapeutic effects in a multi-component, multi-target, and multi-pathway. Finally, a QAMS method capable of simultaneously determining the content of 12 components in WZYZW was established and their quality was analyzed. In conclusion, the Q-marker of WZYZW was preliminarily predicted from three fingerprint, network pharmacology and QAMS as well as could lay the foundation of quality control research of WZYZW. However, the effective constituents, active targets and signaling pathways obtained in research need to be confirmed and validated by animal experiment in further studies.

CRediT authorship contribution statement

Bao-Hua Dong: Writing – original draft, Writing – review & editing. **Jie Wu:** Software, Visualization. **Ying Peng:** Supervision. **Yun-Xiu Jiang:** Supervision. **Ma-Yi-Jie Cao:** Supervision. **Yu Huang:** Investigation, Supervision. **Chang-Jiang Hu:** Funding acquisition, Supervision. **Ling-ying Yu:** Supervision. **Zhi-Min Chen:** Conceptualization, Project administration, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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