Research Article

Multievaluation Strategy for Liujunzi Decoction: Fingerprint Characterization, Chemometrics Analysis, Network Pharmacology, and Molecular Docking

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Liujunzi decoction (LJZD), a traditional tonic formula for treating “qi” deficiency of the spleen and the syndrome of phlegm dampness, can be used to prevent and treat chemotherapy-induced anorexia (CIA). The chemical constituents of LJZD are rather complex; therefore, it is of great significance to establish an effective and economic quality control method to ensure the quality consistency and stability of LJZD. With one chromatographic condition, 13 common peaks detected at 203 nm were selected to establish a fingerprint similarity model and 7 chemical constituents were identified as ephedrine hydrochloride, liquiritin, hesperidin, ginsenoside Rg1, jujuboside A, 6-gingerol, and atracylenolide III. Ten batches of LJZD were divided into two groups by cluster analysis and principal component analysis (PCA), and four main components (ephedrine hydrochloride, hesperidin, ginsenoside Rg1, and jujuboside A) of LJZD were analyzed. Also, the analysis results were combined with network pharmacology and molecular docking technology to further predict how LJZD could prevent and treat CIA. We found that these four main components of LJZD spontaneously combined with four CIA targets (SRC, PIK3R1, MAPK1, and AKT1). In this study, we established the fingerprint of LJZD for the first time, and through a comprehensive multiassesssment method, we also successively analyzed the fingerprint and chemometrics.

1. Introduction

Liujunzi decoction (LJZD) is a traditional formula, which has the effect of enhancing the “qi,” improving spleen function and drying dampness to eliminate phlegm, recorded in the Yi Xue Zheng Zhuan edited by Yu Tuan in the Ming dynasty. It is composed of Ginseng Radix et Rhizoma, Atractylodis Macrocephalae Rhizoma, Poria, Glycyrrhizae Rhizoma et Radix, Citri Reticulatae Pericarpium, Pinelliae Rhizoma, Jujubae Fructus, and Zingiberis Rhiza Recens. LJZD mainly treats body deficiency, gastrointestinal weakness, loss of appetite, fatigue, anemia, and cold limbs, including gastritis, indigestion, loss of appetite, and vomiting. In addition, this formula is also often used to alleviate adverse reactions such as anorexia, nausea, vomiting, and fatigue in patients with cancer after radiotherapy and chemotherapy, which can improve the quality of life and prolong the survival period [1].

Chemotherapy is effective in antineoplastic treatment, but the various side effects, including anorexia, nausea, vomiting, diarrhea, and neurotoxicity, make it difficult for patients to follow the subsequent treatments and result in poor prognosis and quality of life, further limiting the clinical application of chemotherapy [2, 3]. Chemotherapy-induced anorexia (CIA) occurs in 50% of newly diagnosed patients with cancer and up to 70% of patients with advanced diseases [4, 5]. Chinese herbal medicine has unique clinical efficacy, so it is increasingly used as adjuvant therapy for side effects of chemotherapy [6]. In the clinical study of cisplatin-based chemotherapy against lung cancer, Sun et al. and Yoshiya et al. found that LJZD had a definite curative effect in the treatment of CIA, but its mechanism of action
and effective components remained to be explored [7, 8]. Besides, the inadequate quality standards of Chinese medicine formulations or Chinese patent medicines have been considered as one of the reasons for the unstable efficacy [9]. More and more scholars and regulatory officials call for quality standard improvement of Chinese patent medicines, including comprehensive chemical profiles and strict batch consistency [10]. Therefore, the quality standard of LJZD must be higher than that of general Chinese medicine formulas, and the quality standard of the latter is limited to simple chemical qualitative or quantitative analysis [11]. The investigations of LJZD mainly focus on clinical applications and mechanical studies; however, its phytochemical constituents are inadequately demonstrated. Hiroyuki et al. analyzed 32 chemical constituents from LJZD by pharmacokinetic profiles but lacked fingerprint and chemical analyzes [12]. Fingerprint technology of traditional Chinese medicine (TCM) is one of the key technologies in modern Chinese medicine research; thus, in this study we aimed to construct the fingerprint characterization and chemometrics analysis of LJZD for searching main representative components and improving quality control standards.

After years of development, Chinese medicine has formed a unique treatment system and the treatment characteristics of multicomponent synergy [13, 14]. Network pharmacology has sprung up worldwide in recent years. It is based on the theories of system biology, genomics, proteomics, and other disciplines and uses high-throughput omics data analysis, computer simulation, and network database retrieval to reveal the network relationship of drug-gene-target-disease interaction, which can also predict the mechanism of action through network relationship and evaluate the efficacy and adverse reactions of drugs, to research drugs with high efficiency and low toxicity [15]. Molecular docking is a process to find the optimal binding mode between small molecules (ligands) and biological macromolecules (receptors) by simulating the geometric matching and energy matching of molecules through chemometrics methods, including rigid docking, semiflexible docking, and flexible docking [16]. The combination of network pharmacology and molecular docking technology can verify the feasibility of binding predicted compounds with target proteins to increase credibility.

In order to establish a comprehensive, effective, and economic evaluation method of LJZD, we established its fingerprint by HPLC in combination with diversified chemometrics analysis, network pharmacology, and molecular docking and formed a set of methodologies that integrated various technical advantages. In this study, the fingerprint of LJZD was established for the first time, and four main components of LJZD were obtained by chemometrics analysis. Through network pharmacology and molecular docking technology, we found four main components of LJZD, which could spontaneously combine with four main CIA targets such as sarcoma (SRC), phosphatidylinositol 3-kinase regulatory subunit alpha (PIK3R1), mitogen-activated protein kinase 1 (MAPK1), and protein kinase b (AKT1).

2. Materials and Methods

2.1. Chemicals and Materials. LJZD consists of eight TCMs, which were purchased from real estate or main producing areas; no less than 3 producing areas for each herb and no less than 10 batches in total; Table 1S provides the information of these herbal pieces. The standard products of ephedrine hydrochloride, liquiritin, hesperidin, ginsenoside Rg1, jujuboside A, 6-gingerol, and atractylenolide III were purchased from the National Institutes for Food and Drug Control (Beijing, China) and Sichuan Wei-ke-qi Biological Technology Co., Ltd. (Chengdu, Sichuan Province, China), respectively; Table 2S provides their information, and their chemical structures are shown in Figure 1S. Chromatographic pure methanol and acetonitrile were purchased from the Oceanofpak Chemical Firm (Sweden).

2.2. Instruments and Liquid-Phase Conditions. Shimadzu LC-20A high-performance liquid chromatography (SPD-20A/20AV UV detector, Prominence SIL-20A automatic sampler, Shimadzu, Japan) was equipped with LabSolutions CS system (Shimadzu, Japan), the chromatographic column is ZORBAX Eclipse XDB-C18 column (5 μm, 250 mm × 4.6 mm), water (A) and acetonitrile (B) were the mobile phases. The applied gradient was 0–6 min, 5% B; 6–12 min, 5–15% B; 12–35 min, 15–35% B; 35–40 min, 35–55% B; 40–65 min, 55–75% B; 65–80 min, 75–100% B; and 80–85 min, 100% B. The flow rate was 1.0 mL/min. The column temperature was set at 35°C. The injection volume was 10 μL. The standard was weighed by using Sartorius 100,000th balance (SQP SECUAR225D-1CN, Germany).

2.3. Preparation of Standard Solutions. The ephedrine hydrochloride, liquiritin, hesperidin, ginsenoside Rg1, jujuboside A, 6-gingerol, and atractylenolide III were accurately weighed and dissolved in methanol to prepare the mixed standard solution of ephedrine hydrochloride 2.91 mg/ml, liquiritin 0.95 mg/ml, hesperidin 0.98 mg/ml, ginsenoside Rg1 0.65 mg/ml, jujuboside A 1.14 mg/ml, 6-gingerol 2.42 mg/ml, and atractylenolide III 0.93 mg/ml. The mixed standard solution was stored in a refrigerator at 4°C until use.

2.4. Preparation of Sample Solution of LJZD. According to the formula proportion of LJZD (Ginseng : Atractylodis Macrophalae : Poria : Glycyrrhizae : Citri Reticulateae Pericarpium : Pinelliae = 9 : 9 : 6 : 3 : 4.5, plus 2 Jujubes and 3 pieces of Ginger), the appropriate amount of each medicinal material was weighed, soaked for 30 min, and extracted twice. The first time was added 10 times the amount of water for 1 h, and the second time was added 8 times the amount of water for 45 min. The filtrate was combined to obtain the extract of LJZD. The 0.22 μm microporous membrane was used to extract the continuous filtrate, and the LJZD solution (S1–S10) was obtained.
2.5. Statistical Analysis. The liquid-phase data were processed by the LabSolutions CS system. The HPLC fingerprint analysis was performed, automatically corrected, and aligned using the “similarity evaluation system of traditional Chinese medicine chromatographic fingerprint” software (2012.130723 edition, Chinese Pharmacopoeia Commission, Beijing, China), followed by reference/sample fingerprint generation. SPSS 26.0 and SIMCA-P14.1 software (MKS Umetrics, Umea, Sweden) were used for multivariate statistical analysis.

SPSS 23.0 software was used to standardize the peak area of common peaks, the cluster analysis was carried out, and the cluster analysis diagram was drawn by TBioTools software. PCA is based on the principle of data dimension reduction. Principal component analysis can simplify the indicators into representative comprehensive indicators, while maintaining the maximum variance contribution rate of the dataset. In this study, 10 samples were analyzed, and the peak area of their common peaks (13 common peaks) was set as X variable. PCA-X model was selected, and SIMCA-P14.1 was used for principal component analysis. In order to further determine the differential markers, that is, the grouping of LJZD, the OPLS-DA model was established, and the VIP was used to analyze the data matrix. The compound with a VIP value greater than 1 had a general interpretation rate of more than 50% for the classification, which was statistically significant for the classification results, namely, the differential markers.

2.6. Network Pharmacology Analysis. PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used to obtain the molecular formula, CAS number, and 2D SDF files of ephedrine hydrochloride, hesperidin, ginsenoside Rg1, and jujuboside A. Then, the TC MSP database (https://tcmp.e.com/) was used to retrieve the name of the components and check the chemical structural formula to obtain the oral bioavailability (OB) and drug-like (DL) information of the main components of LJZD and to establish the information database of the above four active components.

The SDF format file for each component obtained above was imported into the PharmMapper database (http://www. lilab-ecust.cn/pharmmapper/) to obtain potential targets for the main components of LJZD. The PDB ID number corresponding to the obtained target was transformed by the retrieve/ID mapping function in the UniProt database (https://www.uniprot.org/uploadlists/) to limit the species to human beings and exclude nonhuman targets.

The GeneCards database (https://www.genecards.org/) was searched with the keyword “chemotherapy-induced anorexia” to obtain disease-related targets for the CIA. In this database, the higher the score value, the higher the relevance of the target to the disease. Then, through Venny2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/), the predicted targets of the main components of LJZD obtained above were crossed with the targets of CIA after weight removal.

The intersection targets were imported into the STRING database (https://string-db.org/) to construct PPI network, the species was selected as “homo sapiens,” the score of protein interaction parameters was “high confidence >0.9,” the network display option was “hide disconnected nodes in the network,” and the remaining parameters were unchanged. After running, the PPI network of the main components of LJZD on chemotherapy anorexia was obtained. The analysis results were imported into Cytoscape 3.7.2 software in TSV text format, and the “network analyzer” function in Cytoscape 3.7.2 software was used to analyze the network topology of the analysis results. The degree, betweenness centrality, and closeness centrality of the three important topological parameters were greater than the median, and the target with degree $\geq 10$ was selected as the key target.

Online database DAVID (https://david.abcc.ncifcrf.gov/) was used to perform GO analysis of key module genes, including MF, BP, and CC, and perform KEGG pathway enrichment analysis. MF, BP, CC, and signal pathways with $p$ values less than 0.05 were screened.

Cytoscape 3.7.2 was used to construct the main component-target-pathway network of LJZD by using main components, intersection targets, and important pathways. Among them, four triangular nodes represent the main components of LJZD, the ellipsoids represent the intersection targets, the diamonds represent important pathways, and the edges represent the relationship between components, targets, and pathways.

2.7. Molecular Docking. The 2D structure of small molecular ligands of the main components of LJZD was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and the PubChem CIDs of ephedrine hydrochloride, hesperidin, ginsenoside Rg1, and jujuboside A are 65326, 10621, 441923, and 51346169. The 2D structure was transformed into the 3D structure by ChemOffice software. Chimera 1.15 was used to remove water molecules and small molecular ligands of protein structure and then imported into AutoDockTools for hydrogenation and other pre-treatments. Molecular docking of receptors and ligands was used to analyze their binding and activity.

3. Results

3.1. Optimization of Chromatographic Conditions. The water and acetonitrile were finally selected as mobile phases, column temperature was 35°C, and the flow rate was 1.0 mL/min. In this study, the elution process was one of the most important conditions. On the one hand, the complex peaks needed a fine elution process to separate them. On the other hand, our purpose was to use the same elution process in all experiments. Finally, the gradient elution program was determined, which had the maximum peak capacity, the best peak resolution, and the shortest time. After full-wavelength scanning, 203 nm was selected to establish HPLC fingerprint. The results showed that the wavelength provided the best information presentation and separation and the appropriate peak ratio, as shown in Figure 1. System adaptability data are shown in Table 3S.
3.2. Quality Evaluation of LJZD Based on Fingerprint Similarity and Cluster Analysis. The second step of the evaluation strategy was to give the overall parameters of LJZD to characterize its intrinsic quality and quality differences between different sample batches. Ten batches of LJZD (S1–S10) were analyzed, and the chromatogram at 203 nm was recorded. The chromatographic data (CDF format) were imported into the “similarity evaluation system of traditional Chinese medicine chromatographic fingerprint” software (Ver.2012.130723). With S1 as the reference peak, the average method was used for multipoint correction and chromatographic peak matching to obtain the overlay map, common pattern map, and mixed reference map of 10 batches of LJZD material reference. A total of 13 chromatographic peaks were confirmed, and 7 chromatographic peaks were identified. Figure 2 shows the chromatographic fingerprints of 10 batches of samples. A total of 13 peaks were identified as common peaks. Compared with the reference fingerprint, the similarity of all samples was greater than 0.927 (Table 4S), indicating that the similarity of the reference substance of LJZD was good, the difference of the main material groups was small, and the preparation process was more scientific and reasonable. The reference fingerprint of the LJZD could be used as a standard reference for measuring LJZD.

The quality differences of 10 batches of samples were analyzed by cluster analysis using SPSS23.0 software. The results are shown in Figure 3. Cluster analysis showed that although the similarity of these samples was quite close, the 10 batches of LJZD were roughly divided into two categories: class I was S1–S6, and class II was S7–S10.

3.3. Chemometrics Analysis. The cumulative variance contribution rate of the first three components was 82.698%, indicating that most of the chemical information in the fingerprint of the sample can be reflected by the first three components. The eigenvalue and variance contribution rate are shown in Table 5S. The extracted three principal components represented the vast majority information of 13 common peaks, as shown in Figure 4. The first three components had high slopes, and the main reasons for these differences were mainly caused by different batches of medicinal materials from different habitats.

The initial factor load matrix mainly describes the principal component load on each variable (13 variables in this paper). The higher the load, the stronger the correlation. Three-dimensional loading plot combined with load diagram and rotating ingredients matrix is shown in Figure 5, Table 6S. For example, F1, F2 (ephedrine hydrochloride), F5 (liquiritin), and F7 (hesperidin) were labeled as red. F4, F6, F8 (ginsenoside Rg1), F9, F10 (jujuboside A), and F13 (attractylenolide III) had the largest absolute load on principal component 1, and F1, F2 (ephedrine hydrochloride), F3, F7 (hesperidin), F12 (6-gingerol) had the largest absolute load on principal component 2, and F5 (liquiritin) and F11 mainly explained the information of principal component 3.

In order to produce a more intuitive understanding, we standardized the peak area of common peaks by 0–1 normalization. The results are shown in Figure 6. Consistent with the above analysis results, F1, F2 (ephedrine hydrochloride), F5 (liquiritin), and F7 (hesperidin) were the components with a high correlation in LJZD.

In order to more intuitively reflect the quality of each batch of LJZD, the principal component score and a comprehensive score of 10 batches of LJZD samples were analyzed and compared, and the quality was sorted. The results are shown in Table 7S and Figure 7. According to the comprehensive score ranking, S1–S6 samples can be classified as a class, and S7–S10 can be classified as a single class, which is consistent with the clustering analysis results.

The supervised orthogonal partial least squares discriminant analysis (OPLS-DA) model is used for modeling and analysis, and the components that greatly contribute to the above sample classification are selected. To determine the importance of each variable for identifying and obtaining chemical markers, variable importance in the project (VIP) is used as a significant variable. VIP >1.0 is usually used as a common threshold to measure the contribution of a component to the difference. In this experiment, we found that there were nine peaks whose VIP >1.0. According to the VIP value, they were F1, F7 (hesperidin), F3, F6, F2 (ephedrine hydrochloride), F4, F9, F8 (ginsenoside Rg1), and F10 (jujuboside A); the results are shown in Figure 8. The results were similar to those above, so the four identified active components, namely, F2 (ephedrine hydrochloride), F7 (hesperidin), F8 (ginsenoside Rg1), and F10 (jujuboside A), were used as candidate compounds to match with the chemotherapeutic anorexia targets, and the “component-target-pathway” network was constructed.

3.4. Network Pharmacology and Molecular Docking Verification Analysis. After deleting invalid and repeated targets, a total of 682 targets for the main components of LJZD were obtained, and 143 intersection targets were obtained, which were potential targets for the treatment of CIA by the main components of LJZD; the results are shown in Figure 9. The PPI network is shown in Figure 10. The analysis results were imported into Cytoscape 3.7.2 software, in which twenty-four key targets were screened, and the results are shown in Figure 11. SRC, PIK3R1, MAPK1, and AKT1 were selected as the core targets of molecular docking verification.
A total of 461 gene ontology (GO) items were obtained by GO function enrichment analysis ($p < 0.05$). Among them, the biological process (BP) accounted for 336, the cell composition (CC) accounted for 36, and the molecular function (MF) accounted for 89. The results are shown in Figure 12. CC is mainly involved in intracellular and extracellular processes and focal adhesion. BP mainly includes positive and negative regulations of RNA and DNA transcription, positive and negative regulations of apoptosis, and protein phosphorylation. MF mainly involves enzyme binding, protein kinase activity, adenosine triphosphate binding, zinc ion binding, and transcriptional regulatory region DNA binding. A total of 81 pathways were enriched in the enrichment analysis of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway ($p < 0.05$), and the results are shown in Figure 13. The pathways for the enrichment of main components in LJZD mainly include cancer, PI3K/Akt signaling pathway, proteoglycans in cancer, viral carcinogenicity, hepatitis B, FoxO signaling pathway, and RAS signaling pathway. It can be seen from Figure 14 that the main components of LJZD exert
Figure 6: Heatmap of peak areas from 10 batches of samples by 0-1 normalization.

Figure 7: Principal component analysis score chart.

Figure 8: VIP values of 13 common components.
therapeutic effects on multiple pathways by regulating different protein targets.

As shown in Table 1, the binding energies of the main components of the four LJZD were all less than 0 kJ/mol, indicating that they could spontaneously bind to the target. It can be seen from the results in the table that the main components of LJZD screened above have good binding characteristics with the core target mediators related to CIA.

4. Discussion

LJZD is a traditional Chinese formula that consisted of eight herbs with complex constituents. In order to analyze the chemical constituents as comprehensively as possible, this study investigated the chromatographic conditions and extraction conditions of LJZD. LJZD mainly contains high polar chemical constituents, the column retention properties of these chemical constituents are very similar, and it is difficult to separate them. We tried to use different mobile phases (such as 0.05% phosphoric acid water-acetonitrile, 0.2% phosphoric acid water-acetonitrile, water-methanol, and water-acetonitrile) and ratios, and different column temperatures (such as 25°C, 30°C, and 35°C). Considering the stability and separation efficiency of the sample, the LJZD was filtered by a 0.22 μm microporous membrane and was packed in a 2 ml brown liquid bottle, which was provided in liquid form. By investigating the full wavelength (190 nm – 810 nm), the study found that when phosphoric acid water was used as the aqueous phase, the baseline of the chromatogram seriously shifted downward at the 203 nm selected by the author. When methanol is used as an organic phase, the chromatographic separation effect is not good. When the column temperatures were 25°C or 30°C, multiple components did not appear in the chromatogram. Through the above research, the analysis method of fingerprint of LJZD was established. At present, in the 2020 edition of Chinese Pharmacopoeia, only the content of hesperidin is standardized as the symbol constituent of Liujuanzi Pill that applies in its quality control. Several representative chemical constituents screened in this study can be used as new material reference.

The traditional formula, composed of various herbs, shares the same characteristic of having multiple chemical constituents, targets, and pathways. The compatibility of the classical famous formula pays attention to the compatibility relationship between the monarch and the minister and makes it difficult to study the mechanism of action through the compatibility of the formula. In this experiment, a total of 13 peaks were identified and 7 chromatographic peaks were identified according to the material reference of LJZD. From the number and intensity of chromatographic peaks, Ginseng Radix et Rhizoma, Citri Reticulatae Pericarpium, Pinelliae Rhizoma, and Jujubae Fructus contributed relatively large to the fingerprint, while the remaining Atractylodis Macrocephalae Rhizoma, Poria, Glycyrrhizae Rhizoma et Radi, and Zingiberis Rhizoma Recens contributed less to the fingerprint. In the fingerprint experiment, 13 recognition peaks were identified by cluster analysis, which showed that the original medicinal materials of LJZD from different origins and different batches had differences in fingerprint, which may be related to the growth environment and decoction pieces. For the first time, the fingerprint of the classic famous prescription LJZD was established, and a diversified econometric analysis was carried out to contribute to the multilevel and multifaceted evaluation of LJZD. In addition, the PCA showed the similarity between the samples, but more
detailed information was obtained, such as the representative chemical constituents in LJZD.

By analyzing the main components of LJZD, four representative components were obtained from the above fingerprint combined with PCA analysis. Hesperidin, as a flavonoid compound, has antioxidant activity [17], and anti-inflammatory and antibacterial effects [18]. Hesperidin treatment of CIA may be through its antioxidant, anti-inflammatory, appetizer, and inhibition of IL-6 and NF-κB activities [19]. The other three main components of LJZD also have anti-inflammatory and antitumor effects [20–22], but no research has been found in the treatment of anorexia. The main components of LJZD acted on 24 core targets of CIA, and the targets with a larger degree (SRC, PIK3R1, MAPK1, and AKT1) were analyzed in the literature. Tumor-associated macrophages can produce immunosuppressive factors by activating the Src-RhoA pathway and promoting the proliferation of rectal cancer [23]. Its role in promoting tumor will lead to inflammation in the body and further lead to anorexia during chemotherapy. The miR-574-3p can inhibit proliferation and invasion in esophageal cancer cell by targeting MAPK1, which shows that MAPK1 can be an anti-inflammatory target [24]. PIK3R1 is considered to be...
the downstream gene of miR-92a. It is found in cervical cancer tissues that PIK3R1 is downregulated and negatively correlated with the level of miR-92a. Overexpression of PIK3R1 reverses the promotion of miR-92a on the proliferation, migration, and invasion of cervical cancer, thereby reducing the inflammatory response [25]. The PI3K/Akt signaling pathway is involved in cell proliferation, apoptosis, glucose metabolism, and other physiological activities by activating downstream effectors. Studies have shown that by inhibiting PI3K/Akt signal transduction, oxidative stress and inflammatory factors were reduced [26]. Therefore, the potential therapeutic mechanisms of LJZD in treating CIA may be related to their anti-inflammatory and antioxidative stress effects through interacting with SRC, PIK3R1, MAPK1, AKT1, and other targets. In addition, combined with molecular docking technology, the interaction between components and targets was preliminarily simulated, and the binding energy directly reflects the reliability of the prediction results.

5. Conclusions

In this study, we established the fingerprint for the first time and a comprehensive multiassessment method of LJZD, and the active ingredients including ephedrine hydrochloride, hesperidin, ginsenoside RG1, and jujuboside A were identified as the representative constituents of LJZD. Based on the fingerprint, network pharmacology, and molecular docking, this study analyzed and predicted the efficacy-related substances of LJZD in the treatment of CIA, which laid the foundation for the comprehensive quality control and mechanism research of LJZD compound preparation in the later period. Unfortunately, this study did not verify the efficacy of the targets predicted above via animal experiments, which require further investigations in the future.

### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>AKT1</td>
<td>Protein kinase b</td>
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<tr>
<td>BP</td>
<td>Biological process</td>
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<tr>
<td>CC</td>
<td>Cell composition</td>
</tr>
<tr>
<td>CIA</td>
<td>Chemotherapy-induced anorexia</td>
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<tr>
<td>GO</td>
<td>Gene ontology</td>
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<tr>
<td>KEGG</td>
<td>Kyoto Encyclopedia of Genes and Genomes</td>
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<tr>
<td>LJZD</td>
<td>Liujunzi decoction</td>
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<tr>
<td>MAPK1</td>
<td>Mitogen-activated protein kinase 1</td>
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<td>MF</td>
<td>Molecular function</td>
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OPLS: Orthogonal partial least squares discriminant
DA: analysis
PCA: Principal component analysis
PIK3R1: Phosphatidylinositol 3-kinase regulatory subunit alpha
RSD: Relative standard deviation
S1: Solution 1
SRC: Sarcoma
TCM: Traditional Chinese medicine
VIP: Variable importance in project.

Data Availability
The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Authors’ Contributions
Wu XiPei Xi-Pei Wu conceptualized the study, developed methodology, provided software, and wrote the original draft. Yong-Zhao Dai validated the study, performed formal analysis, provided software, and reviewed and edited the manuscript. Ke Nie contributed to project administration, reviewed and edited the manuscript, and supervised the study.

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Supplementary Materials
Supplementary.pdf includes Figure 1S and Tables 1S–7S. (Supplementary Materials)

References


