Research Article

Network Pharmacology Analysis of Traditional Chinese Medicine Formula Xiao Ke Yin Shui Treating Type 2 Diabetes Mellitus

Jiewen Zhou, Qiuyan Wang, Zhinan Xiang, Qilin Tong, Jun Pan, Luosheng Wan, and Jiachun Chen

Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Huazhong University of Science and Technology, Hangkong Road 13#, Wuhan 430030, China

Correspondence should be addressed to Luosheng Wan; wanluosheng@hust.edu.cn and Jiachun Chen; homespringchen@mail.hust.edu.cn

Received 16 April 2019; Accepted 20 August 2019; Published 8 September 2019

Academic Editor: Srinivas Nammi

Copyright © 2019 Jiewen Zhou et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Xiao Ke Yin Shui (XKYS) formula is a traditional Chinese medicine formula treating type 2 diabetes mellitus (T2DM). XKYS formula consists of four herbs, i.e., Coptidis rhizoma, Liriopes radix, bitter melon, and Cassiae semen. Herein, the chemical profiles of four herb extracts were investigated, and further analysis of the underlying mechanism of XKYS formula treating T2DM was performed using network pharmacology. The main components were selected for our network-based research. Targets of XKYS formula were mainly collected from two databases, SwissTargetPrediction and Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), and the text-mining method was also implemented. T2DM relating genes and therapeutic targets were collected from five databases. Subsequently, STRING and Cytoscape were employed for the analysis of protein-protein interaction (PPI) networks. Functional annotation and pathway analysis were conducted to investigate the functions and relating pathways of target genes. The content of 12 compounds in the herb extracts was determined. With the analysis of PPI networks, a total of 76 genes were found to be important nodes and could be defined as the main target genes regulated by XKYS formula in the treatment of T2DM and its complications. Components in XKYS formula mainly regulate proteins including protein kinase B (Akt), phosphatidylinositol 3-kinase (PI3K), insulin receptor substrate (IRS), and tumor necrosis factor (TNF). XKYS formula exerts therapeutic effects in a synergetic manner and exhibits antidiabetic effect mainly via reducing insulin resistance. These findings could be guidelines in the further investigation of this formula.

1. Introduction

Diabetes mellitus (DM) is now recognized as a complex metabolic disorder, and type 2 diabetes mellitus (T2DM) is the most common type of diabetes [1]. Nowadays, different kinds of therapies are applied in the treatment of diabetes, including insulin and other oral medications. Such therapies may be promising in glycemic control but could also cause side-effects like hypoglycemia or gastrointestinal dysfunction [2]. Thus, more and more people have turned their attention to herbal medicine or diet-based therapies, seeking for safer and more cost-effective complementary medicine for T2DM [3–6].

Traditional Chinese medicine (TCM) is a rich resource, possessing data that can still be hints for the development of new drugs. The focus of the current study, Xiao Ke Yin Shui (XKYS) formula, is recorded in Bencaogangmu (compendium of Materia Medica). XKYS formula contains four herbs, namely, Coptidis rhizoma (dried rhizomes of Coptis chinensis Franch.), Liriopes radix (the dried tuberous roots of Liriope spicata (Thunb.) Lour. var. prolifera Y. T. Ma), bitter melon (the immature fresh fruits of Momordica charantia L.), and Cassiae semen (the dried seeds of Cassia obtusifolia L.). All these herbs are widely used in clinical practice treating diabetes [7].
Several ingredients in these four herbs are generally accepted as the main bioactive components in the treatment of T2DM and its complications, i.e., alkaloids in Coptidis rhizoma, polysaccharides in Liriopes radix, triterpenoids and polysaccharides in bitter melon, and anthraquinone and naphthopyrone in Cassiae semen [8–11]. Thereby, herb extracts were prepared aiming to yield bioactive fractions containing ingredients mentioned above. The mixture of these herb extracts may be a satisfying alternative for the treatment of T2DM.

In XKYS formula, a single component may be responsible for the therapeutic effect through different pathways, and some components may act on the same targets as well. For example, isoquinoline alkaloids from Coptidis rhizoma like berberine are generally recognized as activators of adenosine 5′-monophosphate- (AMP-) activated protein kinase (AMPK) but could also exert therapeutic effects on T2DM through inhibition of protein tyrosine phosphatase 1B (PTP1B) and peroxisome proliferator-activated receptor gamma (PPARγ) [12–14]. Polysaccharides of Liriopes radix and extracts of Cassiae semen could ameliorate glycemic control through activation of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathway [15, 16]. Triterpenoids in bitter melon are proved to be AMPK activators while polysaccharides are inhibitors of PPARγ [17, 18]. These findings, however, could not fully explain the synergetic effects of XKYS formula in the treatment of T2DM and its complications.

Nowadays, researchers have been aware that the “one key, one lock” mode is insufficient to decipher the drug actions, especially in those complex diseases. Network pharmacology, however, analyzing drugs and drug targets in a systemic manner may provide us with novel insights into drug actions [19]. The key ideas of network pharmacology share much with the basic disciplines of TCM, making it a useful tool in the research of TCM [20]. In addition, rapid development of biomedical big data, like TCMSP (Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform), has facilitated such research [21]. Thus, a network-based pharmacological analysis could provide us with a comprehensive understanding towards the significance of each component, target, and pathway.

In brief, this study provided chemical profiles for the extracts of four herbs. And, network pharmacology analysis was applied to understand the underlying mechanisms of XKYS formula in the treatment of T2DM and its complications.

2. Materials and Methods

2.1. Materials and Reagent. Coptidis rhizoma, Liriopes radix, fresh bitter melon, and Cassiae semen were authenticated by Professor Jiachun Chen (School of Pharmacy, Huazhong University of Science and Technology, or HUST). Voucher specimens of these herbs were deposited in Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, HUST.

The four herb extracts were total alkaloids of Coptidis rhizoma (TACR), Liriopes radix polysaccharides (LRP), bitter melon extract (BME), and Cassiae semen extract (CSE), respectively. Detailed preparation methods for the extracts were reported in Supplementary Materials (Part 1).

Acetonitrile (CH3CN) was purchased from Sigma Aldrich (USA). Water was deionized water. Hydrochloric acid, methanol, phosphate buffer, anthrone, sulfuric acid, fructose, glucose, and formic acid (HCOOH) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

HPLC analysis of TACR, BME, and CSE was performed on the Agilent 1260 system with Agilent TC-C18 (250 mm × 4.6 mm, 5 μm) columns. HPLC analysis of LRP was performed on the Hitachi L-2130 system with an Agilent TC-C18 (250 mm × 4.6 mm, 5 μm) column. Content of total carbohydrate was determined on an ultraviolet-visible spectrophotometer, UV-1750 (Shimazu, Japan).

Epiberberine, coptisine, palmatine, berberine, and aurantio-obtusin were purchased from National Institute for Food and Drug Control. Cassiaside, rubifosinarin-6-O-β-D-gentiobioside, glucouranti-obtusin, and cassiaside C were purchased from Chengdu MUST Bio-technology Co. Ltd. (Sichuan, China). Momordicoside L, 7β,25-dihydrocucurbita-5,23(E)-dien-19-α-3-O-β-D-allopyranoside, and momordicoside F2 were self-prepared (Supplementary Materials, Part 2, Figures S1 and S2). The purity of each standard was >98%.

2.2. Analysis of Four Herb Extracts. The analysis of TACR was conducted using the reported method [22].

The total carbohydrate content of LRP was examined using anthrone-sulfuric acid method according to the previous report [23].

Monosaccharide composition analysis method of LRP was performed using the HPLC method after derivatization with 1-pheny-3-methyl-5-pyrazolone (PMP), and the detailed method was reported in Supplementary Materials (Part 3) [24]. The mobile phase was 20:80 (v:v) CH3CN-H3PO4 buffer (pH 7.0). The flow rate was 1.0 ml/min. The injection volume was 20 μl. Column temperature was held constant at 25°C. The UV detection wavelength was 250 nm.

The total carbohydrate content of BME was examined using anthrone-sulfuric acid method according to the previous report [23].

HPLC analysis of BME was conducted with the mobile phase consisting of CH3CN (A) and water (B). The elution program was 0 min 20%A, 10 min 30%A, 30 min 50%A, and 50 min 70%A. The flow rate was 1.0 ml/min. Column temperature was held constant at 25°C. The detector was evaporative light scattering detector (ELSD), with the evaporator temperature at 60°C and nebulizer temperature at 40°C. The flow rate of nitrogen was 1.6 l/min. The standard (10, 20 μl) and sample (20 μl) dissolved in 70% methanol were subjected to HPLC analysis.

HPLC analysis of CSE was carried out according to the previous report [25].

2.3. Construction of Protein-Protein Interaction (PPI) Networks of XKYS Formula Treating T2DM and Its Complications. To identify the corresponding targets of the main components of XKYS formula, several approaches...
combined with chemometric method, information integration, and data-mining were implemented. These components, except polysaccharides, were submitted to TCMSP and SwissTargetPrediction server to find component-target interaction information. TCMSP mainly predicts drug-target interactions with random forest (RF) and support vector machine (SVM) method, while SwissTargetPrediction is a 2D/3D similarity measurement of small molecules [26, 27]. All components, as well as the aglycon of the glycosides, were also submitted to PubMed, SciFinder, and Google Scholar for the text-mining of component-target information. And all targets were submitted to the UniProt database (http://www.uniprot.org) for validation of their gene names. Hereby, a target gene list of XKYS was obtained through in silico investigation.

Another gene list relating to T2DM was established after screening of five databases, including DrugBank database (http://www.drugbank.ca/), Online Mendelian Inheritance in Man (OMIM, http://www.omim.org/), Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.kegg.jp), Therapeutic Target Database (TTD, https://db.idrblab.org/ ttd/), and Text-mined Hypertension, Obesity, and Diabetes Candidate Genes database (T-HOD, http://bws.iis.sinica.edu. tw/THOD/). And all genes and targets were submitted to the UniProt database for validation of their gene names.

Two gene lists were submitted to STRING. The STRING server generated a “Combined Score” ranged from 0 to 1 for each interaction. The higher the score is, the greater the confidence of the interaction. In STRING, an interaction >0.4 means “medium confidence” and >0.7 means “high confidence” [28].

The obtained PPI networks were intersected using Cytoscape 3.2.1, and interactions with score >0.7 were collected, generating a new PPI network, i.e., the PPI network of XKYS formula treating T2DM and its complications.

2.4. Gene Ontology (GO) Functional Annotation and KEGG Pathway Analysis. To elucidate the function of the targets and their role in signaling transduction, the Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to analyze the GO function and KEGG pathway of the main target genes of XKYS formula in the treatment of T2DM and its complications. The biological process, cellular component, molecular function, and the pathway involved were also described.

2.5. Component-Target-Pathway Network Construction. The network model of component-target-pathway was established using Cytoscape 3.2.1. In this network, nodes represent components (C), targets (T), or pathways (P), and edges represent the interaction of C-T or T-P. Based on the results of KEGG enrichment analysis and C-T database, the C-T-P interactions were shown to provide an overview on the mechanisms of XKYS formula in the treatment of T2DM and its complications.

3. Results

3.1. Determination of Main Component Contents of Four Herb Extracts. Four isoquinoline alkaloids, three cucurbitane-type triterpenoids, two anthraquinones, and three naphtopyrones were determined using HPLC. Results of the HPLC analysis are shown in Table 1 and Figure 1, and the structures were also reported (Table 2). The total content of carbohydrate in LRP was determined to be 96.5% while BME 10.8%. The composition of LRP was also determined as fructose:glucose, 20.7:1.

3.2. Screening of Main Components from Four Herb Extracts and Construction of PPI Networks. The screening of 19 components (Table 2) in XKYS had led to the acquisition of 216 targets, while 602 genes relating to the pathophysiology of T2DM were also collected (Supplementary Materials, Tables S1 and S2). All the gene names in two lists were uploaded to STRING, respectively. The XKYS PPI network consists of 216 proteins and 2070 interactions, whereas T2DM, 602 proteins and 11595 interactions (Figures 2(a) and 2(b)). Each interaction has a Combined Score >0.4. By intersecting these two networks, all interactions with high confidence (>0.7) were picked, generating a new PPI network.

The new network consists of 76 genes, representing the main target genes regulated by XKYS formula in the treatment of T2DM. In addition, among these genes, some genes like AKT1, mitogen-activated protein kinase 1 (MAPK1), tumor necrosis factor (TNF) were also found to be important genes involved in the progression of diabetic complications [29–31]. Thus, these genes could also be regarded as the main target genes regulated by XKYS formula in the treatment of T2DM and its complications (Figure 2(c), Table 3).

The new PPI network contains 76 nodes and 333 edges (Figure 2(c)). In this network, nodes represent targets, while edges, the interactions of proteins. And degree, a topological parameter describing the importance of a node, stands for the number of edges connecting to the node. The higher the degree, the more important the target in the network is.

As can be seen in Figure 2(c), important targets were painted red and located centrally in the network. AKT1, PI3KCG, MAPK1, LEP, IRS1, and PPARG were the top six genes regarding their degree.

3.3. GO Functional Annotation and KEGG Pathway Analysis. GO functional annotation was performed on 76 target genes, and the top 20 GO terms (P < 0.01) were selected based on –logP values (Figure 3). GO enrichment analysis indicated that these 76 target genes are responsible for glucose homeostasis, platelet activation, regulation of insulin secretion, cell response to hypoxia, and cellular response to insulin stimulus (Figure 3(a)). These biological processes are related to molecular functions including, steroid hormone receptor activity, protein kinase activity, protein serine/threonine (Ser/Thr) kinase activity, enzyme binding, and drug binding (Figure 3(b)). And, these processes occur mainly in caveola, cytosol, plasma membrane, nucleoplasm, and receptor complex (Figure 3(c)).

KEGG pathway analysis was conducted for further exploration of these targets as shown in Figure 3(d). These
Table 1: Content of components in herb extracts determined using HPLC.

| Extracts | Constituents | Content (mg/g) |
|----------|--------------|----------------|
| TACR     | Epiberberine  | 55.0           |
|          | Coptisine    | 135.1          |
|          | Palmatine    | 99.7           |
|          | Berberine    | 354.7          |
| BME      | Momordicoside L | 1.9    |
|          | 7β,25-Dihydrocucurbita-5,23(E)-dien-19-al3-O-β-D-allopyranoside | 1.0 |
|          | Momordicoside F_2 | 1.9    |
| CSE      | Cassiaside   | 40.0           |
|          | Rubrofusarin 6-O-β-D-gentiobioside | 32.0 |
|          | Glucoaurantio-obtusin | 46.1 |
|          | Cassiaside C | 27.4           |
|          | Aurantio-obtusin | 22.2  |

Figure 1: Continued.
targets were highly enriched in insulin resistance (IR), insulin pathway, adipocytokine pathway, AMPK pathway, T2DM, forkhead box protein O (FoxO) pathway, non-alcoholic fatty liver disease (NAFLD), mammalian target of rapamycin (mTOR) pathway, hypoxia-inducible factor 1 (HIF-1) pathway, glucagon pathway, and so on. These pathways are highly relevant to the development of T2DM and its complications, and XKYS formula may exert therapeutic effects through pathways mentioned above.

3.4. Composition-Target-Pathway Network Construction. A total of 76 genes were defined as the main target genes regulated by XKYS formula in the treatment of T2DM and its complications. These genes were kept, and other entries were omitted in the database of C-T. The combination of C-T and T-P databases had led to a C-T-P network (Figure 4), providing us with an overview on the therapeutic effects of XKYS formula. In addition, the degree of each node is presented in Supplementary Materials Table S3.

The inner cycle represents the main components of XKYS formula. Nodes painted in red were key components interacting with a larger number of targets. Among all of these 19 components, alkaloids from Coptidis rhizoma were regarded as the key components in the treatment of T2DM and its complications.

The middle cycle represents the main target genes regulated by XKYS formula. When painted in red, the corresponding targets are regulated by more components and participate in more pathways. It could be observed that...
Table 2: Structures of components in four herb extracts.

| No. | Name                        | CAS No.   | Molecular formula | Structure | Herb            |
|-----|-----------------------------|-----------|-------------------|-----------|-----------------|
| M1  | Epiberberine                | 6873-09-2 | C_{20}H_{18}NO_{4} | ![Structure](image) | Coptidis rhizoma |
| M2  | Coptisine                   | 3486-66-6 | C_{19}H_{14}NO_{4} | ![Structure](image) | Coptidis rhizoma |
| M3  | Palmatine                   | 3486-67-7 | C_{21}H_{22}NO_{4} | ![Structure](image) | Coptidis rhizoma |
| M4  | Berberine                   | 2086-83-1 | C_{20}H_{18}NO_{4} | ![Structure](image) | Coptidis rhizoma |
| M5  | Liriopes radix polysaccharides | N/A      | N/A               | N/A       | Liriopes radix  |
| M6  | Bitter melon polysaccharides | N/A      | N/A               | N/A       | Bitter melon    |
| M7  | Momordicoside L             | 81348-83-6 | C_{36}H_{58}O_{9} | ![Structure](image) | Bitter melon    |
| M8  | 7β,25-Dihydrocucurbita-5,23(E)-dien-19-al-3-O-β-D-allopyranoside | 912329-04-5 | C_{36}H_{58}O_{9} | ![Structure](image) | Bitter melon    |
| No.  | Name                                      | CAS No.      | Molecular formula | Structure | Herb      |
|------|-------------------------------------------|--------------|------------------|-----------|-----------|
| M9   | 3,7,25-Trihydroxycucurbita-5,23-dien-19-al | 85372-65-2   | C$_{30}$H$_{48}$O$_{4}$ | ![Structure](image) | Bitter melon |
| M10  | Momordicoside F$_2$                       | 81348-82-5   | C$_{36}$H$_{58}$O$_{8}$ | ![Structure](image) | Bitter melon |
| M11  | 5β,19-Epoxycucurbita-6,23-diene-3β,25-diol | 81910-41-0   | C$_{30}$H$_{48}$O$_{3}$ | ![Structure](image) | Bitter melon |
| M12  | Cassiaside                                | 13709-03-0   | C$_{20}$H$_{20}$O$_{10}$ | ![Structure](image) | Cassiae semen |
| M13  | Norrubrofusarin                           | 3566-98-1    | C$_{14}$H$_{10}$O$_{5}$ | ![Structure](image) | Cassiae semen |
| M14  | Rubrofusarin 6-O-β-D-gentiobioside         | 24577-90-0   | C$_{27}$H$_{32}$O$_{15}$ | ![Structure](image) | Cassiae semen |
| M15  | Rubrofusarin                              | 3567-00-8    | C$_{15}$H$_{12}$O$_{5}$ | ![Structure](image) | Cassiae semen |
PI3KR1, PI3KCG, AKT, and TNF are key genes regulated by XKYS formula. This result agreed with that obtained from PPI analysis.

The outer cycle represents the top 20 pathways enriched. Pathways containing the most target genes were in red. IR, AMPK pathway, insulin pathway, and pathway in cancer are the key pathways. This result was familiar to that obtained from KEGG pathway analysis.

4. Discussion

TCM formulae are usually hard to decipher due to its mode of action, namely, “network target, multicomponents” [20]. T2DM is also a complex metabolic disorder with changes in various pathways. XKYS formula and T2DM can be considered as two networks and explained with the help of network pharmacology.

According to C-T-P network, alkaloids from Coptidis rhizoma are the main components in XKYS formula that exert antidiabetic effects. Results also indicated that around 64% of the content in TACR could be determined using the HPLC method. Among these isoquinoline alkaloids determined, berberine accounted for ~35% of the fraction and was also proved to be a key node in the C-T-P network. Berberine has a wide range of biological activities, among which anti-hyperglycemic and anti-hyperlipidemia are two major benefits in the treatment of T2DM [8]. Berberine could activate AMPK via inhibition of mitochondrion respiratory complex I instead of the regulation of calcium/calmodulin-dependent protein kinase kinase beta (CaMKKβ), leading to the reduction of IR and amelioration of lipid metabolism [13, 32]. Momordicoside L and its analogue were detected in BME and interesting enough, and the aglycon of Momordicoside L could activate AMPK through CaMKKβ, which was distinct from the mechanism of berberine [17]. This could be a good evidence of synergetic effect presented by this formula.

LRP are highly enriched according to its total content of carbohydrate. Our previous studies had found that LRP could upregulate the insulin signaling pathway and exert therapeutic effects in diabetic rodents through activation of PI3K/Akt signaling pathway [15, 23]. Aurantio-obtusin, along with its glycosides, accounted for ~6.8% of CSE and was shown to be an important component in the C-T-P network. Previous reports had indicated that aurantio-obtusin may offer therapeutic effects in hypertension through the PI3K/Akt-eNOS (endothelial nitric oxide

| No. | Name                  | CAS No.      | Molecular formula | Structure | Herb            |
|-----|-----------------------|--------------|-------------------|-----------|-----------------|
| M16 | Glucoaurantio-obtusin | 129025-96-3  | C_{23}H_{24}O_{12} | [Image]   | Cassiae semen   |
| M17 | Aurantio-obtusin      | 67979-25-3   | C_{17}H_{14}O_{7}  | [Image]   | Cassiae semen   |
| M18 | Cassiaside C          | 119170-52-4  | C_{27}H_{32}O_{15} | [Image]   | Cassiae semen   |
| M19 | Toralactone           | 41743-74-2   | C_{15}H_{12}O_{5}  | [Image]   | Cassiae semen   |

N/A = not applicable.

---

Table 2: Continued.
synthase) pathway, implying a possible mechanism in the treatment of diabetic complications of XKYS formula [33].

As can be seen in the C-T-P network, AKT1, PI3KCG, PI3KR1, and TNF were key genes regulated by XKYS formula in the treatment of T2DM and its complications. Proteins expressed by genes AKT1, PI3KCG, and PI3KR1 were all important members in the signaling transduction of PI3K/Akt. This signaling pathway is of great significance in the progression of T2DM due to its role in glucose metabolism [34]. The PI3K/Akt signaling pathway is also responsible for the regulation of the signaling pathway like MAPK, FoxO, and nuclear factor kappa B (NF-κB). These pathways play important roles in the regulation of protein synthesis, cell survival, differentiation, proliferation, and apoptosis and are highly related to the proliferation and regeneration of islet β-cells [29].

Figure 2: Protein-protein interaction networks. The mapping of PPI network was generated by the STRING server. (a) PPI network of genes regulated by XKYS formula (217 nodes, 2070 edges). (b) PPI network of genes relating to the pathophysiology of T2DM (602 nodes, 11595 edges). (c) 76 main target genes regulated by XKYS formula in the treatment of T2DM and its complications. This network contains 76 nodes and 333 edges. As shown in the color bar, nodes in red could be considered as important and nodes in green are less important in this network. The degree value of each node in Figure 2(c) is presented in Table 3.
### Table 3: Main targets regulated by XKYS formula in the treatment of T2DM and its complications.

| Protein name                                                                 | Gene name | Unipro ID  | PPI network degree |
|------------------------------------------------------------------------------|-----------|------------|--------------------|
| RAC-alpha serine/threonine-protein kinase                                   | AKT1      | P31749     | 27                 |
| Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit gamma isoform | PIK3CG    | P48736     | 24                 |
| Insulin receptor substrate 1                                               | IRS1      | P35568     | 22                 |
| Leptin                                                                       | LEP       | P41159     | 22                 |
| Mitogen-activated protein kinase 1                                         | MAPK1     | P28482     | 22                 |
| Peroxisome proliferator activated receptor gamma                           | PPARG     | P37231     | 21                 |
| Tumor necrosis factor                                                       | TNF       | P01375     | 20                 |
| Glucagon                                                                    | GCG       | P01275     | 17                 |
| Prostaglandin G/H synthase 2                                                | PTGS2     | P35354     | 17                 |
| Nitric-oxide synthase, endothelial                                          | NOS3      | P29474     | 16                 |
| Phosphatidylinositol 3-kinase regulatory subunit alpha                       | PIK3R1    | P27986     | 16                 |
| Apoptosis regulator Bcl-2                                                  | BCL2      | P10415     | 15                 |
| Estrogen receptor                                                           | ESR1      | P03372     | 15                 |
| Insulin receptor                                                            | INSR      | P06213     | 15                 |
| Mitogen-activated protein kinase 14                                         | MAPK14    | Q16539     | 15                 |
| Mitogen-activated protein kinase 3                                          | MAPK3     | P27361     | 15                 |
| Interleukin-4                                                               | IL4       | P05112     | 14                 |
| Peroxisome proliferator-activated receptor alpha                            | PPARA     | Q07869     | 13                 |
| Peroxisome proliferator-activated receptor gamma coactivator 1-alpha        | PPARGC1A  | Q9UBK2     | 13                 |
| Heme oxygenase 1                                                            | HMOX1     | P09601     | 11                 |
| Solute carrier family 2, facilitated glucose transporter member 4           | SLC2A4    | P14672     | 11                 |
| C-C motif chemokine 2                                                       | CCL2      | P13500     | 10                 |
| Prothrombin                                                                 | F2        | P00734     | 10                 |
| Matrix metalloproteinase-9                                                  | MMP9      | P14780     | 10                 |
| Protein kinase C delta type                                                 | PRKCD     | Q05655     | 10                 |
| NAD-dependent protein deacetylase sirtuin-1                                 | SIRT1     | Q96EB6     | 10                 |
| Sterol regulatory element-binding protein 1                                 | SREBF1    | P36956     | 10                 |
| Androgen receptor                                                           | AR        | P10275     | 9                  |
| Leptin receptor, LEP-R                                                      | LEPR      | P48357     | 9                  |
| Phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-1             | PLCG1     | P19174     | 9                  |
| Protein kinase C alpha type                                                 | PRKCA     | P17252     | 9                  |
| Protein kinase C beta type                                                  | PRKCB     | P05771     | 9                  |
| Tyrosine-protein phosphatase nonreceptor type 1                             | PTPN1     | P18031     | 9                  |
| Retinoic acid receptor RXR-alpha                                            | RXRA      | P19793     | 9                  |
| Acetyl-CoA carboxylase 1                                                    | ACACA     | Q13085     | 8                  |
| Tumor necrosis factor ligand superfamily member 6                           | FASLG     | P48023     | 8                  |
| Protein kinase C epsilon type                                               | PRKCE     | Q02156     | 8                  |
| Glycogen synthase kinase-3 beta                                            | GSK3B     | P49841     | 7                  |
| Inhibitor of nuclear factor kappa-B kinase subunit beta                     | IKBKB     | O14920     | 7                  |
| Nitric oxide synthase, inducible                                           | NOS2      | P35228     | 7                  |
| 5′-AMP-activated protein kinase catalytic subunit alpha-1                    | PRKAA1    | Q13131     | 7                  |
| Glucose-6-phosphatase                                                       | G6PC      | P35575     | 6                  |
| 5-Hydroxytryptamine receptor 2C (by homology)                               | HTR2C     | P28335     | 6                  |
| 5′-AMP-activated protein kinase catalytic subunit alpha-2                    | PRKAA2    | P54646     | 6                  |
| Mitochondrial brown fat uncoupling protein 1                                | UCP1      | P25874     | 6                  |
| Beta-2 adrenergic receptor                                                  | ADRB2     | P07550     | 5                  |
| Glucokinase                                                                 | GCK       | P35557     | 5                  |
| 5-Hydroxytryptamine receptor 2A (by homology)                               | HTR2A     | P28223     | 5                  |
| 5-Hydroxytryptamine receptor 2B                                             | HTR2B     | P41595     | 5                  |
| Phosphoenolpyruvate carboxykinase, cytosolic (GTP)                          | PCK1      | P35558     | 5                  |
| Peroxisome proliferator-activated receptor delta                            | PPARD     | Q03181     | 5                  |
| Adenosine receptor A1                                                       | ADORA1    | P30542     | 4                  |
| Alpha-2A adrenergic receptor (by homology)                                  | ADRA2A    | P08913     | 4                  |
| Alpha-2B adrenergic receptor                                                | ADRA2B    | P18089     | 4                  |
Table 3: Continued.

| Protein name                                      | Gene name  | Unipro ID  | PPI network degree |
|---------------------------------------------------|------------|------------|--------------------|
| Alpha-2C adrenergic receptor                       | ADRA2C     | P18825     | 4                  |
| D(2) dopamine receptor (by homology)              | DRD2       | P14416     | 4                  |
| Hepatocyte nuclear factor 1-alpha                 | HNF1A      | P20823     | 4                  |
| Mitogen-activated protein kinase 10               | MAPK10     | P53779     | 4                  |
| Glucocorticoid receptor                           | NR3C1      | P04150     | 4                  |
| Acyl-CoA desaturase                                | SCD        | O00767     | 4                  |
| Solute carrier family 2, facilitated glucose transporter member 1 | SLC2A1 | P11166 | 4 |
| Mitochondrial uncoupling protein 2                | UCP2       | P55851     | 4                  |
| Carnitine O-palmitoyltransferase 1, liver isoform | CPT1A      | P50416     | 3                  |
| D(1A) dopamine receptor                           | DRD1       | P21728     | 3                  |
| 3-Hydroxy-3-methylglutaryl-coenzyme A reductase   | HMGCR      | P04035     | 3                  |
| Prostacyclin receptor                             | PTGIR      | P43119     | 3                  |
| Superoxide dismutase (Cu-Zn)                      | SOD1       | P00441     | 3                  |
| Aldose reductase                                   | AKR1B1     | P15121     | 2                  |
| Cyclin-dependent-like kinase 5                    | CDK5       | Q00535     | 2                  |
| Aromatase                                         | CYP19A1    | P11511     | 2                  |
| Corticosteroid 11-beta-dehydrogenase isozyme 1    | HSD11B1    | P28845     | 2                  |
| Transcription factor AP-2-alpha                   | TFAP2A     | P05549     | 2                  |
| Vitamin D3 receptor                                | VDR        | P11473     | 2                  |
| Angiotensin-converting enzyme                      | ACE        | P12821     | 1                  |
| Dipeptidyl peptidase 4                            | DPP4       | P27487     | 1                  |
| Prostaglandin G/H synthase 1                       | PTGS1      | P23219     | 1                  |

(a) Biological process

(b) Molecular function
Tumor necrosis factor (TNF) is a cytokine that could be secreted by macrophages and adipose cells. It can induce IR by downregulation of the activity of PI3K/Akt signaling pathway. It has also been reported that MAPK and NF-κB signaling pathways are stimulated by TNF and further regulates inflammatory response, oxidative stress, and apoptosis [31].

As shown in KEGG pathway analysis, 23 out of 76 targets were found to participate in IR, ranking no. 1 according to its −log P value. Other pathways on the KEGG list were adipocytokine pathway, AMPK pathway, insulin pathway, T2DM, FoxO pathway, NAFLD, etc. And, this finding was further approved by the C-T-P network. All of these signaling pathways are all highly related to IR. Thus, we could make a preliminary inference from this result that the most important mechanism of XKYS formula in the treatment of T2DM may be reducing IR.

IR is a condition in which the target organs are insensitive to insulin. Long-term of unhealthy diet and lacking exercises could cause overweight or even obesity. Overweight or obese patients are in metabolic disorder states with excessive fat accumulation [35]. Some experts had put forward a hypothesis that obesity is a chronic condition of inflammation [36]. Adipocytokines like TNF and interleukin-6 (IL-6) could inhibit the binding of insulin receptor (InsR) and insulin receptor subunit (IRS). IRS would be degraded under such conditions. In addition, inflammation response and oxidative stress interact as both cause and effect, causing the damage of islet β-cells and diabetic microangiopathy and then leading to the onset of T2DM and its complications [37]. Moreover, the activity of AMPK in skeletal muscles and liver reduces in IR conditions, causing decreased oxidation of free fatty acids and a lower intake of glucose, which in turn deteriorating glycemic control [38]. The FoxO pathway is one of those key factors in the transition from IR to the damage of islet β-cells. The dysfunction of β-cells is caused by various factors, including oxidative stress and inflammatory response. The FoxO pathway is highly related to the risk factors mentioned above [39].

In addition, the pathway in cancer was also an important pathway enriched in the C-T-P network (Figure 4). For one thing, diabetes is a risk factor for carcinogenesis due to hyperinsulinemia, hyperglycemia, and fat-induced chronic inflammation [40]. For another, components in XKYS formula like isoquinoline alkaloids and cucurbitane-type triterpenoids were reported to possess antitumor activities through pathways participating in the pathophysiology of...
both diabetes and cancer [41, 42]. Thus, such correlation was also presented in C-T-P network.

It should also be noted that the PI3K/Akt signaling pathway is also regarded as a key regulator in the development of diabetic complications [29]. And, among those pathways enriched, the hypoxia-inducible factor 1 (HIF-1) pathway and vascular endothelial growth factor (VEGF) pathway are considered to be important factors involved in the development of diabetic complications [43, 44].

The network-based pharmacological analysis has shown that XKYS formula could regulate the glucose and lipid metabolism and alleviate IR mainly through insulin, AMPK, adipocytokine, and FoxO pathway. And, this formula could also be used in the treatment of diabetic complications due to its effect on PI3K/Akt, HIF-1, and VEGF pathways.

This study provided an overview on the antidiabetic effects of XKYS formula in a holistic manner. However, in vivo and in vitro experiments are required to offer more information about the mechanisms of XKYS formula.

5. Conclusion

Components in XKYS formula mainly regulate proteins including Akt, PI3K, IRS, and TNF. XKYS formula exerts therapeutic effects in a synergetic manner and exhibits antidiabetic effect mainly via reducing IR. These findings could be guidelines in the further investigation of this formula.

Abbreviations

| Term      | Description                                      |
|-----------|--------------------------------------------------|
| Akt       | Protein kinase B                                 |
| AMPK      | Adenosine 5’-monophosphate (AMP) activated protein kinase |
| BME       | Bitter melon extract                             |
| CaMKKβ    | Calcium/calmodulin-dependent protein kinase beta |
| CSE       | Cassiae Semen extract                            |
| DM        | Diabetes mellitus                                |
| FoxO      | Forkhead box protein O                           |
| GO        | Gene ontology                                    |
| HIF-1     | Hypoxia-inducible factor 1                       |
| InsR      | Insulin receptor                                 |
| IR        | Insulin resistance                               |
| IRS       | Insulin receptor subunit                         |
| KEGG      | Kyoto encyclopedia of genes and genomes          |
| LRP       | Liriopis radix polysaccharides                   |
| mTOR      | Mammalian target of rapamycin                    |
NAFLD: Nonalcoholic fatty liver disease
NF-κB: Nuclear factor kappa B
PI3K: Phosphatidylinositol 3-kinase
PPARγ: Peroxisome proliferator-activated receptor gamma
PPI: Protein-protein interaction
T2DM: Type 2 diabetes mellitus
TACR: Total alkaloids of Coptidis rhizoma
TCM: Traditional Chinese medicine
TCMSP: Traditional Chinese medicine systems pharmacology database and analysis platform
TNF: Tumor necrosis factor
VEGF: Vascular endothelial growth factor
XKYS: Xiao Ke Yin Shui formula.

Data Availability

The data used to support the findings of this study are included within the article and the Supplementary Materials.

Conflicts of Interest

The authors declare that there are no existing conflicts of interest.

Acknowledgments

The authors greatly appreciate the financial support from the National Key R&D Program-Research Projects on Modernization of Traditional Chinese Medicine (No. 2017YFC1701000), Major Technological Innovation Projects in Hubei Province (No. 2016ACA141), and National Natural Science Fund of China (No. 81773869).

Supplementary Materials

Figure S1: structures of M7, M8, and M10. Figure S2: HPLC spectra of M7 (A), M8 (B), and M10 (C) and bitter melon extract (D). Table S1: in silico screening results of XKYS. Table S2: the gene list collected from online resources relating to T2DM. Table S3: information of each node in the component-target-pathway network. (Supplementary Materials)

References

[1] D. J. Magliano, P. Zimmet, and J. E. Shaw, “Classification of diabetes mellitus and other categories of glucose intolerance,” in International Textbook of Diabetes Mellitus, R. A. DeFronzo, E. Ferrannini, P. Zimmet, and K. G. M. M. Alberti, Eds., pp. 1-16, Wiley-Blackwell, Hoboken, NJ, USA, Fourth edition, 2015.

[2] American Diabetes Association, “Pharmacologic approaches to glycemic treatment: standards of medical care in diabetes-2019,” Diabetes Care, vol. 42, no. 1, pp. S90–S102, 2019.

[3] X.-L. Tong, L. Dong, L. Chen, and Z. Zhen, “Treatment of diabetes using traditional Chinese medicine: past, present and future,” The American Journal of Chinese Medicine, vol. 40, no. 5, pp. 877–886, 2012.

[4] M. S. H. Akash, K. Rehman, and S. Chen, “Effects of coffee on type 2 diabetes mellitus,” Nutrition, vol. 30, no. 7-8, pp. 755–763, 2014.

[5] M. S. H. Akash, K. Rehman, and S. Chen, “Spice plant Allium cepa: dietary supplement for treatment of type 2 diabetes mellitus,” Nutrition, vol. 30, no. 10, pp. 1128–1137, 2014.

[6] M. S. H. Akash, K. Rehman, M. Tariq, and S. Chen, “Zingiber officinale and type 2 diabetes mellitus: evidence from experimental studies,” Critical Reviews in Eukaryotic Gene Expression, vol. 25, no. 2, pp. 91–112, 2015.

[7] Diabetes Society of China Association of Chinese Medicine, “Criteria on the diagnosis and treatment of diabetes mellitus in traditional Chinese medicine,” World Journal of Integrated Traditional and Western Medicine, vol. 6, no. 6, pp. 540–547, 2011.

[8] H. Wang, W. Mu, H. Shang, J. Lin, and X. Lei, “The anti-hyperglycemic effects of Rhizoma Coptidis and mechanism of actions: a review of systematic reviews and pharmacological research,” BioMed Research International, vol. 2014, Article ID 798093, 10 pages, 2014.

[9] J. Fang, X. Wang, M. Lu, X. He, and X. Yang, “Recent advances in polysaccharides from Ophiopogon japonicus and Lirope spicata var. prolifera,” International Journal of Biological Macromolecules, vol. 114, pp. 1257–1266, 2018.

[10] S. Jia, M. Shen, F. Zhang, and J. Xie, “Recent advances in Momordica charantia: functional components and biological activities,” International Journal of Molecular Sciences, vol. 18, no. 12, p. 2555, 2017.

[11] X. Dong, J. Fu, X. Yin et al., “Cassiae Semen: a review of its phytochemistry and pharmacology,” Molecular Medicine Reports, vol. 16, no. 3, pp. 2331–2346, 2017.

[12] C. Huang, Y. Zhang, Z. Gong et al., “Berberine inhibits 3T3-L1 adipocyte differentiation through the PPARγ pathway,” Biochemical and Biophysical Research Communications, vol. 348, no. 2, pp. 571–578, 2006.

[13] Y. S. Lee, W. S. Kim, K. H. Kim et al., “Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states,” Diabetes, vol. 55, no. 8, pp. 2256–2264, 2006.

[14] C. Chen, Y. Zhang, and C. Huang, “Berberine inhibits PTP1B activity and mimics insulin action,” Biochemical and Biophysical Research Communications, vol. 397, no. 3, pp. 543–547, 2010.

[15] Y. Liu, L. Wan, Z. Xiao, J. Wang, Y. Wang, and J. Chen, “Antidiabetic activity of polysaccharides from tuberous root of Liriope spicata var. prolifera in KKAY mice,” Evidence-Based Complementary and Alternative Medicine, vol. 2013, Article ID 349790, 11 pages, 2013.

[16] M. L. Zhang, X. Li, H. F. Liang et al., “Senen Cassiae extract improves glucose metabolism by promoting GLUT4 translocation in the skeletal muscle of diabetic rats,” Frontiers in Pharmacology, vol. 9, p. 235, 2018.

[17] T. J. Iseli, N. Turner, X.-Y. Zeng et al., “Activation of AMPK by bitter melon triterpenoids involves CaMKKβ,” PLoS One, vol. 8, no. 4, Article ID e62309, 2013.

[18] X. Y. He and Z. H. Liu, “Study on HTS anti-diabetic agents from Momordica charantia L.,” Experimental studies,” Critical Reviews in Eukaryotic Gene Expression, vol. 25, no. 2, pp. 91–112, 2015.

[19] A. L. Hopkins, “Network pharmacology,” Nature Biotechnology, vol. 25, no. 10, 1110–1111, 2007.

[20] S. Li and B. Zhang, “Traditional Chinese medicine network pharmacology: theory, methodology and application,” Chinese Journal of Natural Medicines, vol. 11, no. 2, pp. 110–120, 2013.
[21] J. Ru, P. Li, J. Wang et al., “TCMSP: a database of systems pharmacology for drug discovery from herbal medicines,” Journal of Cheminformatics, vol. 6, no. 1, p. 13, 2014.

[22] Chinese Pharmacopoeia Commission, Pharmacopoeia of the People’s Republic of China 2015, Part 1, Chemical Industrial Press, Beijing, China, 2015.

[23] Z.-Q. Xiao, Y.-L. Wang, S.-R. Gan, and J.-C. Chen, “Polysaccharides from Liriodendron Radix ameliorates hyperglycemia via various potential mechanisms in diabetic rats,” Journal of the Science of Food and Agriculture, vol. 94, no. 5, pp. 975–982, 2014.

[24] J. Dai, Y. Wu, S.-W. Chen et al., “Sugar compositional determination of polysaccharides from Dunaliella salina by modified RP-HPLC method of precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone,” Carbohydrate Polymers, vol. 82, no. 3, pp. 629–635, 2010.

[25] Q. Wang, J. Zhou, Z. Xiang et al., “Anti-diabetic and renoprotective effects of Cassiae Semen extract in the streptozotocin-induced diabetic rats,” Journal of Ethnopharmacology, vol. 239, article 111904, 2019.

[26] H. Yu, J. Chen, X. Xu et al., “A systematic prediction of multiple drug-target interactions from chemical, genomic, and pharmacological data,” PLoS One, vol. 7, no. 5, Article ID e37608, 2012.

[27] D. Gfeller, A. Grosdidier, M. Wirth, A. Daina, O. Michielin, and V. Zoete, “SwissTargetPrediction: a web server for target prediction of bioactive small molecules,” Nucleic Acids Research, vol. 42, no. W1, pp. W32–W38, 2014.

[28] D. Szklarczyk, J. H. Morris, H. Cook et al., “The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible,” Nucleic Acids Research, vol. 45, no. D1, pp. D362–D368, 2017.

[29] I. Hers, E. E. Vincent, and J. M. Tavaré, “Akt signalling in health and disease,” Cellular Signalling, vol. 23, no. 10, pp. 1515–1527, 2011.

[30] M. Toyoda, D. Suzuki, M. Honma et al., “High expression of PKC-MAPK pathway mRNAs correlates with glomerular lesions in human diabetic nephropathy,” Kidney International, vol. 66, no. 3, pp. 1107–1114, 2004.

[31] W.-M. Chu, “Tumor necrosis factor,” Cancer Letters, vol. 328, no. 2, pp. 222–225, 2013.

[32] N. Turner, J.-Y. Li, A. Gosby et al., “Berberine and its more biologically available derivative, dihydroberberine, inhibit mitochondrial respiratory complex I: a mechanism for the action of berberine to activate AMP-activated protein kinase and improve insulin action,” Diabetes, vol. 57, no. 5, pp. 1414–1418, 2008.

[33] S. Li, Q. Li, X. Lv et al., “Aurantio-obtusin relaxes systemic arteries through endothelial PI3K/AKT/eNOS-dependent signaling pathway in rats,” Journal of Pharmacological Sciences, vol. 128, no. 3, pp. 108–115, 2015.

[34] J. A. Engelman, J. Luo, and L. C. Cantley, “The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism,” Nature Reviews Genetics, vol. 7, no. 8, pp. 606–619, 2006.

[35] A. R. Martins, R. T. Nachbar, R. Gorjao et al., “Mechanisms underlying skeletal muscle insulin resistance induced by fatty acids: importance of the mitochondrial function,” Lipids in Health and Disease, vol. 11, no. 1, p. 30, 2012.

[36] P. A. Tataranni and E. Ortega, “A burning question: does an adipokine-induced activation of the immune system mediate the effect of overnutrition on type 2 diabetes?,” Diabetes, vol. 54, no. 4, pp. 917–927, 2005.

[37] K. Rehman and M. S. H. Akash, “Mechanism of generation of oxidative stress and pathophysiology of type 2 diabetes mellitus: how are they interlinked?,” Journal of Cellular Biochemistry, vol. 118, no. 11, pp. 3577–3585, 2017.

[38] G. L. Russo, M. Russo, and P. Ungaro, “AMP-activated protein kinase: a target for old drugs against diabetes and cancer,” Biochemical Pharmacology, vol. 86, no. 3, pp. 339–350, 2013.

[39] T. Kitamura, “The role of FoxO1 in β-cell failure and type 2 diabetes mellitus,” Nature Reviews Endocrinology, vol. 9, no. 10, pp. 615–623, 2013.

[40] J. Wojciechowska, W. Krajewski, M. Bolanowski, T. Kręcicki, and T. Zatoński, “Diabetes and cancer: a review of current knowledge,” Experimental and Clinical Endocrinology & Diabetes, vol. 124, no. 5, pp. 263–275, 2016.

[41] Y. Pan, F. Zhang, Y. Zhao et al., “Berberine enhances chemosensitivity and induces apoptosis through dose-orchestrated AMPK signaling in breast cancer,” Journal of Cancer, vol. 8, no. 9, pp. 1679–1689, 2017.

[42] J.-R. Weng, L.-Y. Bai, C.-F. Chiu, J.-L. Hu, S.-J. Chiu, and C.-Y. Wu, “Cucurbitane triterpenoid from Momordica charantia induces apoptosis and autophagy in breast cancer cells, in part, through pereoxisome proliferator-activated receptor γ activation,” Evidence-Based Complementary and Alternative Medicine, vol. 2013, Article ID 935675, 12 pages, 2013.

[43] H. Takahashi and M. Shibuya, “The vascular endothelial growth factor (VEGF)/VEGF receptor system and its role under physiological and pathological conditions,” Clinical Science, vol. 109, no. 3, pp. 227–241, 2005.

[44] S.-B. Catrina, “Impaired hypoxia-inducible factor (HIF) regulation by hyperglycemia,” Journal of Molecular Medicine, vol. 92, no. 10, pp. 1025–1034, 2014.