Research Article

Systematic Elucidation of the Mechanism of Oroxylum indicum via Network Pharmacology

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Oroxylum indicum (O. indicum) is an important traditional Chinese medicine that exerts a wide spectrum of pharmacological activities. However, the pharmacological effect of O. indicum and its mechanism of action have not to be systematically elucidated yet. In this study, the druggability for active compounds of O. indicum was assessed via Traditional Chinese Medicine Systems Pharmacology Database (TCMSP), and the potential drug targets of O. indicum were identified using PharmMapper database. Additionally, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed via WebGestalt. Drug-target-pathway networks were constructed using Cytoscape to give a visual view. Our findings revealed that O. indicum has extremely superb druggability with 41 putative identified target genes. GO, KEGG, and network analyses showed that these targets were associated with inflammatory immunoreactions, cancer, and other biological processes. In summary, O. indicum is predicted to target multiple genes/proteins and pathways that shape a network which can exert systematic pharmacological effects.

1. Introduction

Traditional Chinese medicine (TCM) is the most plentiful source of bioactive compounds or pharmaceutical components for drug development [1]. Oroxylum indicum (O. indicum), for instance, is an important herbal medicine that belongs to the Bignoniaceae family [2] and has been extensively used for centuries in many Asia countries [3]. It is known as Mu Hu Die [4] in China and also found throughout South and Southeast Asian countries such as Japan, Thailand, and India [5, 6]. Oroxylum indicum belongs to the Bignoniaceae family [2] and possesses a wide spectrum of pharmacological effects such as antioxidant [4], antimicrobial [7], antiallergic [8], anti-inflammatory [9], and anticancer [10–12] properties.

Attention has been closely paid to Oroxylum indicum owing to its underlying roles in the prevention and clinical therapy of a wide spectrum of diseases, for instance, cough, asthma, pertussis, pharyngitis, chronic or acute bronchitis, arthritic and rheumatic problems, and tumors [2, 3, 12]. These findings suggest that Oroxylum indicum could be used as a valuable TCM by reason of the incision of complicated pathophysiological processes, identification of therapeutic targets, and discovery of potential molecular functions and involved pathways. However, the possible molecular mechanisms that Oroxylum indicum induces are rarely investigated. Simultaneously, utilization of computational approaches to identify the drug target molecules and uncover the hidden mechanisms is becoming the main current for the sake of saving time, money, and effort. Especially important, computational target identification and the following molecular mechanisms could speed up the progress of drug development.

Therefore, we systematically investigated the pharmacological functions of Oroxylum indicum with the employment of computational approaches. Firstly, the active
compounds of *Oroxylum indicum* were screened and evaluated using the Traditional Chinese Medicine Systems Pharmacology (TCMSP) resource. Next, the potential target genes of those active compounds were predicted by the PharmMapper database. Moreover, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted with the utilization of the predicted targets. Finally, the drug-targets and targets-pathways pharmacological data of *Oroxylum indicum* were integrated and further used to construct a three-layer network. An overview of the analytical procedures for *Oroxylum indicum* target gene identification and mechanism investigation is illustrated in Figure 1(a).

### 2. Materials and Methods

#### 2.1. Evaluation of Oral Bioavailability and Druglikeness by TCMSP

TCMSP (http://lsp.nwu.edu.cn/tcmsp.php) is a systematic database of pharmacology for natural compounds or TCM [13]. It provides information on the absorption, distribution, metabolism, and excretion (ADME) activities of compounds or TCMs, such as druglikeness (DL), oral bioavailability (OB), and blood-brain barrier (BBB) [14, 15].

Of all the ADME-related properties, DL and OB are the most vital features of administered drugs, due to their roles in evaluating the efficacy of the drug distribution to the circulatory system, and how drug-like a compound is with respect to factors like bioavailability. In the TCMSP database, DL was calculated based on the Tanimoto coefficient and molecular descriptors, while OB was evaluated on OBioavail 1.1 based on an in-house model [13, 14].

In this study, “*Oroxylum indicum*” was input to TCMSP database, and its pharmacokinetics activities were assessed at the molecular level. Compounds with OB ≥ 40% and DL ≥ 0.2 were screened for further investigation.

#### 2.2. Target Identification via PharmMapper Database

PharmMapper (http://www.lilab-ecust.cn/pharmmapper/) is an online reverse-docking database for potential target identification of small molecules [16]. Given a small molecule in Mol2 or SDF format, it provides the top 300 targets sorted by the normalized fit score in descending order. In the present study, the Mol2 format files of the active compounds identified were downloaded from TCMSP and uploaded individually to the PharmMapper. Only human protein targets were chosen and other arguments were set to default values. The top 10 targets identified of the individual compound were selected for further investigation.

#### 2.3. GeneMANIA Analysis

GeneMANIA (http://www.genemania.org) is an online tool for generating hypotheses concerning gene function, analyzing gene sets, and prioritizing genes for functional assays [17].

Given a query gene list, GeneMANIA can discover functionally similar genes based on curated genomics and proteomics data. After selecting *Homo sapiens* from the organism option, the target gene list of interest was input to the search box, and the results were further collated.

#### 2.4. Gene Function and Pathway Enrichment Analyses

We employed a Web-based gene set analysis toolkit (WebGestalt, http://www.webgestalt.org/option.php) to systematically investigate the functions and pathway enrichment information on the target genes we predicted [18]. The gene of interest was entered into WebGestalt web server utilizing overrepresentation enrichment analysis (ORA) approach with Gene Oncology [19] and KEGG databases [20, 21] and other default parameters. False discovery rate (FDR) adjusted *p* value less than 0.05 was considered to be significant statistically.

#### 2.5. Network Construction

For comprehensively understanding the complex association among the drug, target gene, and related pathways, we used Cytoscape (v 3.7.2) to build and analyze the drug-targets-pathways networks.

### 3. Results

#### 3.1. Screening of Active Compounds from TCMSP

TCMSP provides detailed information on vital ADME-related activities such as human oral bioavailability (OB) and druglikeness (DL). The active compounds of *Oroxylum indicum* were identified based on the ADME-related properties from the TCMSP database, with the threshold OB ≥ 40% and DL ≥ 0.2. 10 active compounds were yielded and finally chosen for further investigation (Figure 1(b) and Table 1).

#### 3.2. Identification of Potential Drug Targets

Potential targets for the 10 active compounds of *Oroxylum indicum* were identified using PharmMapper [16] with selecting the top 10 targets of individual compound based on the normalized fit score. We obtained their official gene symbol and gene ID from PDB and UniProt and the Gene database of the National Center for Biotechnology Information (NCBI), yielding 41 targets after the removal of duplicates. After the removal of the duplicates, we finally identified 41 unique target genes of the 10 active compounds (Table 2). These 41 identified target genes were utilized for further investigation.

#### 3.3. GeneMANIA Analysis

Among the 41 target genes and their interacting genes, it was uncovered that 40.32% displayed coexpression characteristics, 31.43% had physical interactions, and 12.96% engaged in colocalization. Other results, including shared protein domains, pathway, and genetic interactions, are illustrated in Figure 2.

#### 3.4. GO and KEGG Pathway Analyses

To further understanding the 41 predicted targets, GO and KEGG enrichment analyses were performed by applying WebGestalt. As demonstrated in Figure 3, the top seven functions were metabolic process (40/41), biological regulation (38/41), protein binding (37/41), response to stimulus (33/41), cell
communication (27/41), multicellular organismal process (25/41), and membrane-enclosed lumen (25/41). These GO terms were highly relevant to anti-inflammatory activities, particularly for pharyngitis, chronic or acute bronchitis, and other respiratory diseases. As for the pathway analysis, we found the 41 target genes participated in 10 KEGG pathways with significant FDR adjusted p value including pathways in cancer, complement and coagulation cascades, and apoptosis (Figure 4).

3.5. Network Analysis. Based on target identification and pathway analysis, an entire drug, targets, and involved pathways network was built via Cytoscape (v 3.7.2). As demonstrated in Figure 5, this three-layer network had 52 nodes and 86 edges. The red oblong, green invert triangles, and blue circles represent drug, target genes, and related pathways, respectively.

4. Discussion

Traditional Chinese medicine (TCM) has been applied extensively to prevent and treat various kinds of diseases owing to its high efficiency, no drug resistance, and low toxicity [22, 23]. Thus, the development of active compounds derived from TCM on the drug design and discovery process should be prioritized urgently [24–27]. In silico analyses can improve pharmacokinetic modeling, prediction, as much as toxicity, and metabolic endpoints; all of which streamline and speed up the drug development progress [28, 29].

Identification of target genes is the first step in drug discovery. It has been revealed that more and more active
drugs or compounds interact with multiple genes or proteins to exert their pharmacological functions [30–33]. Different kinds of *in silico* target identification approaches have been developed and are widely used towards this end. As shown in Table 2, 41 potential target genes of *Oroxylum indicum* were identified using computational approaches. GeneMANIA analysis, with information on coexpression, physical interactions, and colocalization, as well as pathway, shared protein domains, and genetic interactions, indicated that these target genes and their interacting genes may have identical or similar functions.

Accordingly, we identified an inflammatory role for *Oroxylum indicum* in pharyngitis and chronic or acute bronchitis. For example, the majority of the identified targets of *Oroxylum indicum* were enriched in biological regulation, response to stimulus, cell communication, and so on. These were all essential for *Oroxylum indicum* to exert its inflammatory role. Similarly, Begum et al. also found *Oroxylum indicum* plays a vital role in inhibiting inflammation, antiulcerative, enhancing immunity, and lowering blood glucose [6]. These findings coincide closely with our results via GO and KEGG analyses. Furthermore, we discovered that some of the predicted target genes of *Oroxylum indicum* were associated with cancer, such as EGFR, PIM1, ESR1/2, and MAPK8/10. These genes are closely related to cell proliferation, differentiation, or migration and participate in pathways in cancer, complement and coagulation cascades, apoptosis, signaling pathway, and so on. More importantly, some of these are transcription factors that coregulate several pathways simultaneously, like EGFR and ESR1/2. Consistently, Li et al. have also discovered that *O. indicum* induces apoptosis via PI3K/Akt/PTEN signaling pathway in liver cancer [12]. These interesting findings may elucidate the potential

| No. | Gene ID | Gene symbol | Target name |
|-----|---------|-------------|-------------|
| 1   | 5292    | PIM1        | Proto-oncogene serine/threonine-protein kinase Pim-1 |
| 2   | 3055    | HCK         | Tyrosine-protein kinase HCK |
| 3   | 2099    | ESR1        | Estrogen receptor |
| 4   | 590     | BCHE        | Cholinesterase |
| 5   | 2147    | F2          | Prothrombin |
| 6   | 5241    | PGR         | Progesterone receptor |
| 7   | 1956    | EGFR        | Epidermal growth factor receptor |
| 8   | 7276    | TTR         | Transthyretin |
| 9   | 760     | CA2         | Carbonic anhydrase 2 |
| 10  | 2629    | GBA         | Glucosylceramidase |
| 11  | 1646    | AKR1C2      | Aldo-keto reductase family 1 member C2 |
| 12  | 9261    | MAPKAPK2    | MAP kinase-activated protein kinase 2 |
| 13  | 650     | BMP2        | Bone morphogenetic protein 2 |
| 14  | 412     | STS         | Steryl-sulfatase |
| 15  | 213     | ALB         | Serum albumin |
| 16  | 4129    | MAOB        | Amine oxidase [flavin-containing] B |
| 17  | 336     | APOA2       | Apolipoprotein A-II |
| 18  | 7068    | THR8        | Thyroid hormone receptor-beta |
| 19  | 840     | CASP7       | Caspase-7 |
| 20  | 4314    | MMP3        | Stromelysin-1 |
| 21  | 5599    | MAPK8       | Mitogen-activated protein kinase 8 |
| 22  | 5602    | MAPK10      | Mitogen-activated protein kinase 10 |
| 23  | 759     | CA1         | Carbonic anhydrase 1 |
| 24  | 1017    | CDK2        | Cell division protein kinase 2 |
| 25  | 2950    | GSTP1       | Glutathione S-transferase P |
| 26  | 5142    | PDE4B       | cAMP-specific 3,5-cyclic phosphodiesterase 4B |
| 27  | 1675    | CFD         | Complement factor D |
| 28  | 6256    | RXRA        | Peroxisome proliferator-activated receptor-gamma |
| 29  | 7376    | NR1H2       | Oysterole receptor LXR-beta |
| 30  | 6626    | SNRPA       | U1 small nuclear ribonucleoprotein A |
| 31  | 2159    | F10         | Coagulation factor X |
| 32  | 25824   | PRDX5       | Peroxiredoxin-5, mitochondrial |
| 33  | 23173   | METAP1      | Methionine aminopeptidase 1 |
| 34  | 5478    | PPIA        | Peptidyl-prolyl cis-trans isomerase A |
| 35  | 283     | ANG         | Angiogenin |
| 36  | 2100    | ESR2        | Estrogen receptor-beta |
| 37  | 23621   | BACE1       | Beta-secretase 1 |
| 38  | 6678    | SPARC       | SPARC |
| 39  | 7048    | TGFB2       | TGF-beta receptor type-2 |
| 40  | 5836    | PYGL        | Glycogen phosphorylase, liver form |
| 41  | 629     | CFB         | Complement factor B |

Table 2: Drug targets for active compounds of *Oroxylum indicum.*
mechanism of antitumor role that *Oroxylum indicum* exerts.

The drug-targets network illustrated in Figure 5 also demonstrated that *Oroxylum indicum* has multiple targets to exert multiple pharmacological effects. Multiple target therapeutic drugs are more effective for the treatment of complex diseases, such as cough, asthma, pertussis, and cancers, and are less vulnerable to drug resistance. Therefore,
Oroxylum indicum could be a promising resource that may be used as a lead compound, chemical moiety, or active ingredients for further drug development. Nevertheless, we have to acknowledge that there are some limits and bias in our analyses due to the databases we employed, for example, the release, update date, and resources they used.

5. Conclusion

In short, we would like to emphasize that Oroxylum indicum is a valuable TCM and contains quite promising compounds for the development of an effective and safe multitargeted anti-inflammatory and antitumor medicament. This study
provides novel insight into the perspectives and challenges as for the Oroxylum indicum research and clinical application in future investigations.

**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 no conflicts of interest in this work.

**Authors’ Contributions**

Junmin Chen and Jianyong Chen contributed equally to this work.

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