The Prediction of Active Ingredients and Polypharmacological Mechanisms of *Orthosiphon stamineus Benth.* (Lamiaceae) of Dai medicine based on Network Pharmacology

Xingqiang Wang[1,2], Weiqing Zhao[3], Xiaoyu Zhang[1], Zongqing Wang[1], Chang Han[1], Jiapeng Xu[4], Guohui Yang[5], Jiangyun Peng[1,2*], Zhaofu Li[1*]

[1] Department of Rheumatology, The No.1 Affiliated Hospital of Yunnan University of Traditional Chinese Medicine, Kunming, Yunnan, 650021, PR China

[2] Yunnan Provincial Clinical Medicine Research Center, Yunnan, 650021, PR China

[3] Department of Rheumatology and Immunology, The First People's Hospital of Yunnan Province and The Affiliated Hospital of Kunming University of Science and Technology, Kunming, Yunnan, 650034, PR China

[4] Department of Yi Medicine, Traditional Chinese Medicine Hospital of Chuxiong Yi Autonomous Prefecture (Traditional Yi medicine hospital of Yunnan Province), Chuxiong, Yunnan, 675000, PR China

[5] Department of Medical Research Information, Traditional Chinese Medicine Hospital of Chuxiong Yi Autonomous Prefecture (Traditional Yi medicine hospital of Yunnan Province), Chuxiong, Yunnan, 675000, PR China

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Background: *Orthosiphon stamineus Benth.* (OS) was a traditional folk herb with widespread clinical application, but it was lack of comprehensive understanding of its active ingredients and polypharmacological mechanisms. The aim of this work was to systematically study its natural compounds and the molecular mechanisms of the OS via network pharmacology.

Methods: Compounds from OS were collected by literature retrieval and evaluated by SwissADME with the physicochemical property (ADMET model) and the likelihood for a natural medicine. The connection of active ingredients and target genes was built and confirmed by Cytoscape and AutoDock vina. Then, Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were separately performed to obtain more in-depth understanding of OS. Finally, the relationship among active ingredients, targets, and diseases was built to clarify the polypharmacological mechanisms and found relevant active substances for further drug discovery.

Results: A total of 140 compounds of OS were collected, and 16 potentially active ingredients were screened out. The Ingredient-Target Interaction Network was built with 6 flavonoids, 3 diterpenes, 3 phenols, 4 volatile oils, and 64 targets. The Docking analysis indicated that the ingredient-target interaction network was reliable; most ligand-receptor had a strong binding affinity (lowest binding energy: -6.9 kcal/mol). After pathway analysis, 185 significant biological processes and 36 signal pathways were found, and the ingredient-target-disease network of OS was constructed for polypharmacological mechanisms.

Conclusion: Our study clarified the polypharmacological mechanisms via the relationship among active ingredients, targets, and diseases and provided better guidance for subsequent experiments and potential active ingredients for drug discovery.

Keywords: Orthosiphon stamineus, network pharmacology, water-soluble ingredient, Orthosiphol A, Rosmarinic acid, Eupatorin, mechanisms, polypharmacology

1 INTRODUCTION

In traditional folk medicine, *Orthosiphon stamineus Benth.* (Lamiaceae), also termed *Clerodendranthus spicatus (Thunb.) C.Y.Wu* in China, is a widely consumed medical plant known as *misai kucing*, *cat's whiskers*, *Java tea, kumis kucing,* and others all over the world[1, 2]. *O. stamineus* is extensively distributed in tropical or subtropical areas such as Southeast Asia and North Australia and used as herbal tea, drinks and a decoction of Dai medicine[3]. *O. stamineus,* also named "ya nu miao" (Pinyin) in Dai language, is considered a high medicinal value that was consistently applied to treat nephritis, cystitis, rheumatism, edema, hypertension, diabetes, obesity, hepatitis, and so on. In addition, many pharmacopeias such as Indonesian, Malaysia, Dutch, and French, have listed the therapeutic indications: renal cleansing and tonic kidney, used for the treatment of calculus and weight loss. And it is even employed to treat tumors and urinary tract infections in some countries[4].

Although the widespread application of *O. stamineus* in the clinical practice of folk medicine, it is still insufficient to support its curative effect. Plenty of pre-clinical experiments demonstrated that the various extracts of *O. stamineus* exerted pharmacological properties, including anti-proliferative[5, 6], antioxidant[7, 8], analgesic[9], anti-inflammatory[8, 10], antimicrobial[11, 12], diuretic[13], among others. Recently, two rare clinical trials have been performed, and the results showed that the herbal combination with *O. stamineus* seems to be useful in the reduction of discomfort and prevention of antibiotic use in women with urinary tract infections[14, 15]. Despite the fact that dozens of phytochemical studies investigated the natural compounds in *O. stamineus* from all over the world and hundreds of chemical compounds such as flavones, polyphenols, and terpenoids have been detected, very little is known about active substances, their targets, interactions, and specific molecular mechanisms. Thus, it continues to be fierce challenges for us to systematically study these natural compounds and the molecular mechanisms, which benefit further trials and drug discoveries.

Network pharmacology, a novel analytical method for traditional Chinese medicine (TCM) that has emerged in the last decade, could integrate systems biology, disease knowledge, polypharmacology, and herbal medicine to conduct a systematic analysis on human diseases under the guidance of a holistic perspective[16, 17]. It is common knowledge that the traditional herb or herbal formulae ameliorate health via multicomponent interaction with multiple targets, regulation of multiple biological processes, and synergistic effects. This view shifts the traditional paradigm of "one disease, one target, one drug" to develop a "network-target, multicomponent therapeutics" mode[18, 19]. Therefore, now the network pharmacology is a very appropriate technology to explore herbs of Dai medicine systematically.

In view of the shortcomings of the current researches of *O. stamineus*, in our study, we scrutinized related studies and collected almost all compounds of *O. stamineus.* Then, we evaluated the physicochemical property, oral bioavailability, and drug-

likeness for the accumulation of phytochemical information by network pharmacology tools. Finally, the relationship among active ingredients, targets, and diseases was built to clarify the polypharmacological mechanisms and found relevant active substances for further drug discovery.

2 MATERIAL AND METHODS

The workflow of this study was depicted in Figure 1.

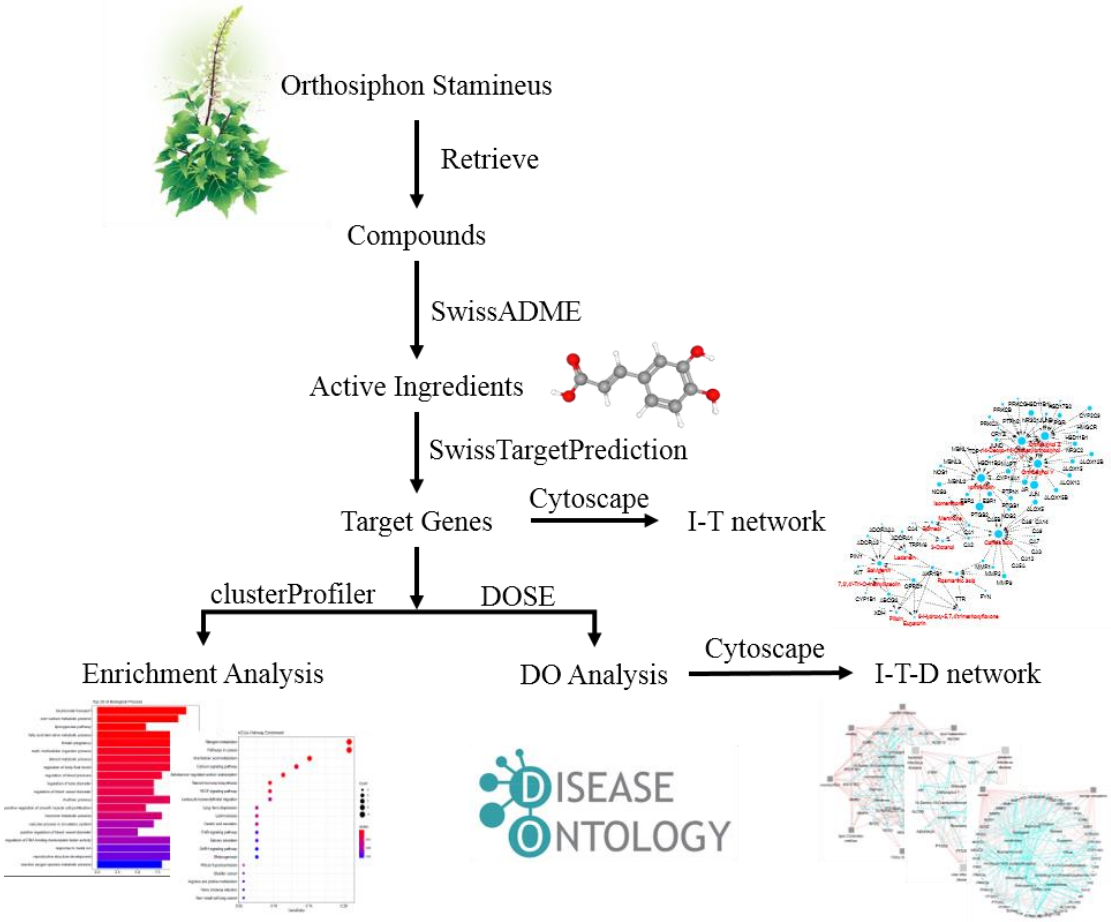

Figure 1 Diagram of methodology applied in this study.

2.1 Compounds Database Building and ADMET Evaluation

In order to collect the information of compounds from various extracts of Orthosiphon stamineus, we retrieved literatures in PubMed, Embase, google scholar, and Chinese databases including CNKI (http://new.oversea.cnki.net/), WANFANG data (http://www.wanfangdata.com.cn/), and SinoMed (http://www.sinomed.ac.cn/). The key words were including “Orthosiphon stamineus”, “clerodendranthus spicatus”, “Java tea”, and “compound”. Then got the compound CID, SMILES identifier of compounds collected via PubChem (https://pubchem.ncbi.nlm.nih.gov/) and constructed compounds database of Orthosiphon stamineus by Microsoft Excel 2013. ADMET is an acronym in pharmacology for absorption, distribution, metabolism, excretion, and toxicity, and it plays an important role in drug discovery. Here, we conduct a web tool SwissADME (http://www.swissadme.ch/) to evaluate ADMET

parameters and drug-likeness[20].

2.2 Screening Potential Active Ingredients

SwissADME provided many chemical properties of small molecules including water solubility, gastrointestinal absorption, drug-likeness (DL) and oral bioavailability (OB)[20, 21]. Orthosiphon stamineus was usually made as decoction to intake, so we screened the potential active ingredients via the following indicators: (i) Log S > -6 or Solubility class was at least moderately soluble, (ii) the compound must fully met Lipinski‘s Rule of Five[22], (iii) and OB ≥ 30%.

2.3 Target Genes and Protein-Protein Interaction Network Prediction

Based on SMILES identifier, target genes of each potential active ingredients were predicted by SwissTargetPrediction (http://www.swisstargetprediction.ch/) with “Homo sapiens” setting[23]. The probability in prediction output page was calculated from the combined-score of the compound most similar to the query molecule with a given protein as target[24]. The greater the probability is, the more accurate the predicted target is. We set a probability of 0.2 as the threshold to filter more credible target genes.

To obtain protein-protein interaction network, the filtered target genes were inputted into STRING (https://string-db.org/) with “Homo sapiens” as organism, and protein-protein interaction network was obtained by setting “minimum required interaction score” as “high confidence (0.700)” during conducting STRING[25].

2.4 Gene Function and Pathway Enrichment Analysis

In order to better understand the biological effects of active ingredients to target genes, gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were separately performed using R package “clusterProfiler”[26]. The qvalue was the corrected P value by Benjamini-Hochberg (BH) method and it was significant for qvalue less than 0.05.

2.5 Disease Ontology Analysis

The Disease Ontology (DO) is a standardized ontology of human disease for the examination and comparison of genetic variation, phenotype, protein, drug and epitope data (https://disease-ontology.org/)[27]. Based on it, DO analysis aim to analyze which human diseases (or class of diseases) are enriched in my gene or protein sets by hypergeometric tests. Aim at target genes above, we performed the DO analysis via R package “DOSE”[28]. The qvalue was the corrected P value by Benjamini-Hochberg (BH) method and it was significant for qvalue less than 0.05.

2.6 Network Construction

The ingredient-target-disease (I-T-D) network was established via Cytoscape 3.7.1 through three steps as follows[29]: (i) the active ingredient-target network was generated by linking the potential active ingredients, target genes and protein-protein interaction; (ii) the target-disease network was constructed by extracting the results of

DO analysis; (iii) the effects or relationships from active ingredient to disease were analyzed by segmenting the I-T-D network into subnetworks based on disease classification. In these results, nodes stand for active ingredients, target genes or diseases, and edges represent the interaction of the three. So the degree value of node is the edge numbers of ingredients or genes in network, and it represents the importance of the ingredients or genes.

2.7 Molecular Docking Analysis

To validate the reliability of the ingredient-target associations, we performed molecular docking analysis using AutoDock vina 1.1.2 (http://vina.scripps.edu/index.html)[30]. Here, we only take some hub target genes and ingredients for example. Firstly, the ingredients (ligands) were downloaded from PubChem database with SDF format and the target proteins (receptors) was collected from RCSB web site (http://www.rcsb.org/) in PDB format. Then the SDF files of ligands were converted into PDB format and the original crystal ligands, water molecules were removed from receptors. As preparation of docking, both the receptors and the ligands were added hydrogens, computed Gasteiger charges, and the non-polar hydrogens were merged. The docking site was defined within a grid box around residues of the binding site and the grid space was set as 1 Å. Finally the AutoDock vina 1.1.2 were performed to explore the conformational binding site and compute binding energies using the Lamarckian genetic algorithm, and the other parameters (e.g. random seed was set as 708076952 and number of iterations was set to100 times) were the same for each docking.

2.8 Statistical Software

The statistical analysis was performed by using R 3.5.0 (2018/04/23), Bioconductor 3.7, MGLTools 1.5.6 (http://mgltools.scripps.edu/), AutoDock vina 1.1.2, and Cytoscape 3.7.1 on windows system x64.

3 RESULTS

3.1Potential Active Ingredients

Through literature retrieval and evaluation, we collected 140 compounds, including terpenoids, flavonoids, aromatics, phenols, alkyls, organic acid and so on. These compounds were all listed in Supplementary table s1. After the calculating in web tool SwissADME, we screened out 52 potential active ingredients (listed in Supplementary table s2) applying to the screen criteria above, Particularly, volatile oils account for 35 of the 52 active ingredients in the orthosiphon aristatus (about 67.31%), terpenoids account for 19 (36.54%), flavonoids account for 7 (13.46%), indicating that these three chemicals were the main active ingredients in orthosiphon aristatus.

3.2 the ADME Parameters and Physicochemical Properties of These Ingredients

As shown in Supplementary table s2, the average molecular weight of these ingredients was about 205.15, the median was 155.26, the maximum was 492.56, and the minimum was 100.16. The prediction results of drug characteristics shown that of the 52 active

ingredients, 88.5% had good water solubility, 96.2% had good intestinal absorption and 82.7% can permeate blood brain barrier (BBB). There were 28 small molecules predicted as non-inhibitor of five major cytochromes P450 (CYP) isoforms. All the 52 active ingredients had high OB and DL values, for example, the OB value of 50 compounds was 55% and 2 compounds was 56%. All the 52 compounds complied fully with "Lipinski's rule of five", indicated that these had great drug-likeness. These results demonstrated that *orthosiphon aristatus* was reasonable as a decoction to intake.

3.3 Ingredient-Target Interaction Network

In order to understand Ingredient-target (I-T) interaction mechanisms in human body, we connected potential active ingredients with target proteins or genes via conducting SwissTargetPrediction and STRING. In the results, a total of 64 target genes (proteins) linked to 16 active ingredients as shown in Supplementary table s3. We used 16 potential active constituents and relevant targets to generate a bipartite graph that illustrated interaction between compounds and its target proteins, target proteins and target proteins (Figure 2). The resultant I-T network contains 80 nodes and 208 edges, including 6 flavonoids, 3 diterpenes, 3 phenols, and 4 volatile oils. In this I-T network, 14-Deoxo-14-O-acetylorthosiphol Y, Orthosiphol Y, Orthosiphol Z, vomifoliol and Caffeic acid each were surrounded with 15 target proteins, and were identified as the center of network to suggested which importance in pharmaceutical effect. There were some targets shared multiple constituents were considered as hub target proteins, such as AKR1B1 was a target of 6 compounds, ABCG2 and OPRD1 all were the targets of 5 flavonoids. Additionally, 4 essential oils only map to 4 targets depicted in Figure 2 and it was insignificant for *orthosiphon aristatus* to cure disease, due to which were mostly present in fresh plants and were easily volatile during processing.

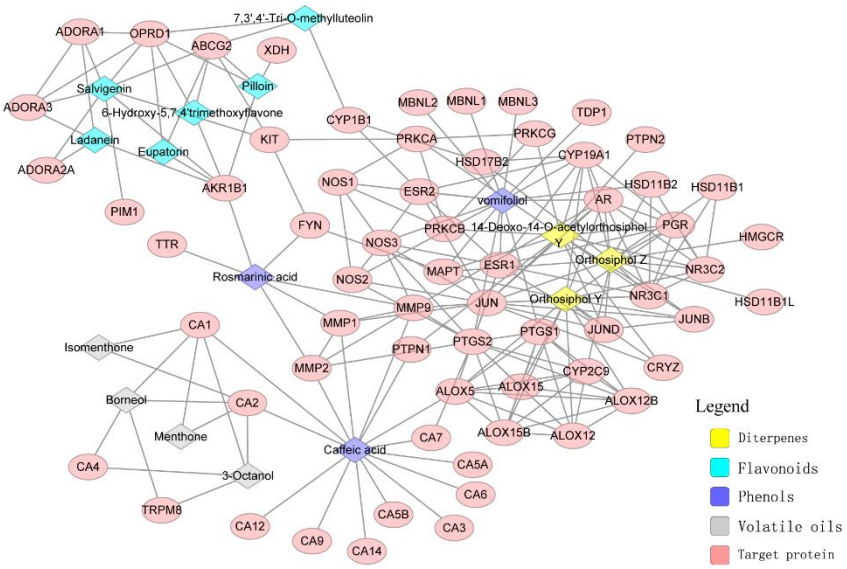

Figure 2 The ingredient-target interaction network of *orthosiphon aristatus.* The pink ellipses represent target genes and the colorful diamonds stand for potential active constituents in *orthosiphon aristatus*.
Target names are shown inrectangles

3.4 molecular docking analysis

There were 39 pairs of ligand-receptor complex were analyzed to access the relative binding affinity of ligand toward receptor and predict the binding sites in our silico study. Based on the resultant of docking analysis shown in table s4, the binding free energies ranged from -6.9 kcal/mol to -16.3 kcal/mol. The hub target AKR1B1 had 6 ligands 6-Hydroxy-5,7,4'trimethoxyflavone, Salvigenin, Eupatorin, Pilloin, Ladanein and Rosmarinic acid, their binding energies respectively were -10.9, -11.3, -11.5, -12.1, -11.9 and -13.2 kcal/mol. Particularly, Orthosiphol Z may bind to many targets such as AR, NR3C1, PGR and it all have highest binding affinity (docking score: -14.5 to -16.3). In brief, these docking simulations indicated that the ingredient-target interaction network was of reliability and most ligand-receptor had a strong binding affinity.

3.5 potential mechanism

In this section, we input 64 target genes into "clusterProfiler" for biological effects of 12 active ingredients. The results of GO enrichment analysis and KEGG pathway enrichment analysis were shown in Supplementary table s5 and table s6.There were 185 significant biological process and 36 signal pathways to be found, and we selected the top 20 terms (ranked via adjust P value) for charting. As figure 3A, the potential drug targets was major related to human biological process with "bicarbonate transport", "arachidonic acid metabolic process", "long-chain fatty acid biosynthetic process", "fatty acid derivative metabolic process", "hormone metabolic process", "regulation of blood pressure", "reactive oxygen species metabolic process" and so on. The 36 signal pathways listed in table s5 such as "Nitrogen metabolism", "Arachidonic acid metabolism", "Aldosterone-regulated sodium reabsorption", "VEGF signaling pathway" could related with potential pharmacological dynamic mechanism of *orthosiphon aristatus*(figure 3B).

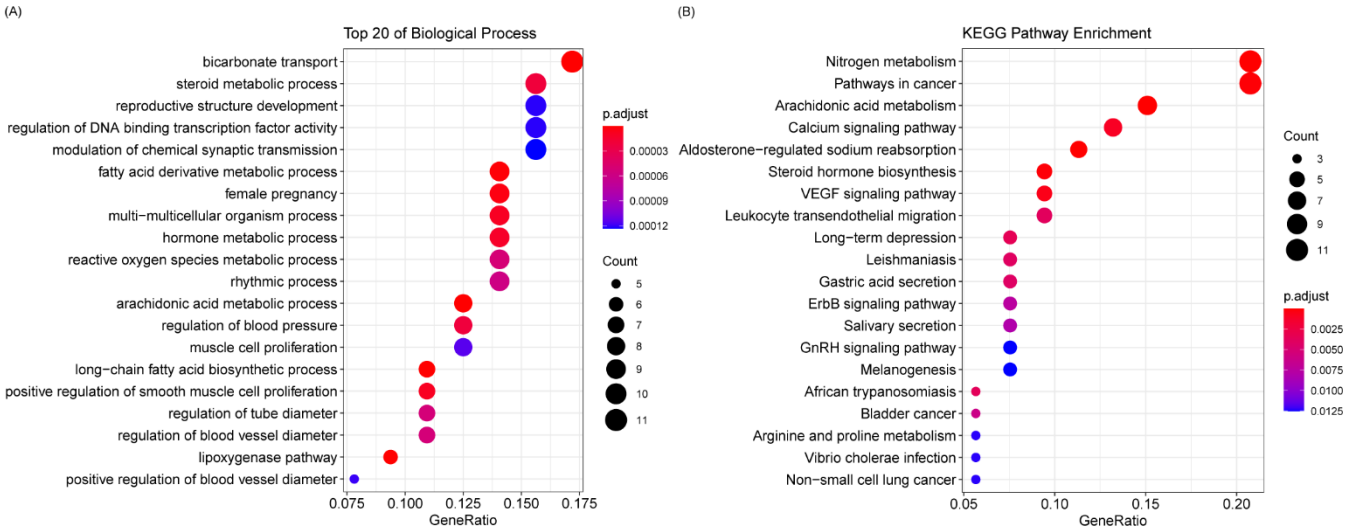

Figure 3 The dot plot of enrichment with target genes.

3.6 Unraveling the molecular basis of diseases treated by *orthosiphon aristatus*.

orthosiphon aristatus, a valued traditional folk medicine, has been widely used for

several human disease or conditions including edema, hypertension, nephritis, diabetes, hyperlipidemia, urinary inflammation, calculus, gout, rheumatism etc., because their diuretic, antioxidative, anti-inflammatory, analgesic, cytotoxic, antihypertensive, hypolipidemic property. Here, we connected drug-target network with diseases by DO analysis, since it could be an effective way to global understand the *orthosiphon aristatus*. Assuming that drug components acting on the same protein in the same network can be linked to multiple diseases and treat these diseases finally, this disease-centric network may provide more evidence and novel sight on applying traditional medicine.

3.6.1 Regulating metabolism

The annotation by DO analysis show that of the 16 active ingredients, 15 linked to some nutritional and metabolic diseases, which main contained type 2 diabetes mellitus, lipid metabolism disorders (familial hyperlipidemia, hyperglycemia and Fabry disease) and nutritional disease (obesity, overnutrition). Inspect of the figure 4, there are 21 targets related to the nutritional and metabolic diseases, and the hub target proteins NR3C1, AKR1B1, PGR, ALOX12, PTGS2 and NOS3 had the most degree in this network and were tightly connected with lipid metabolism disorders and obesity. It depicted the orthosiphon aristatus could treat these metabolic diseases through regulating of these targets (figure 4A).

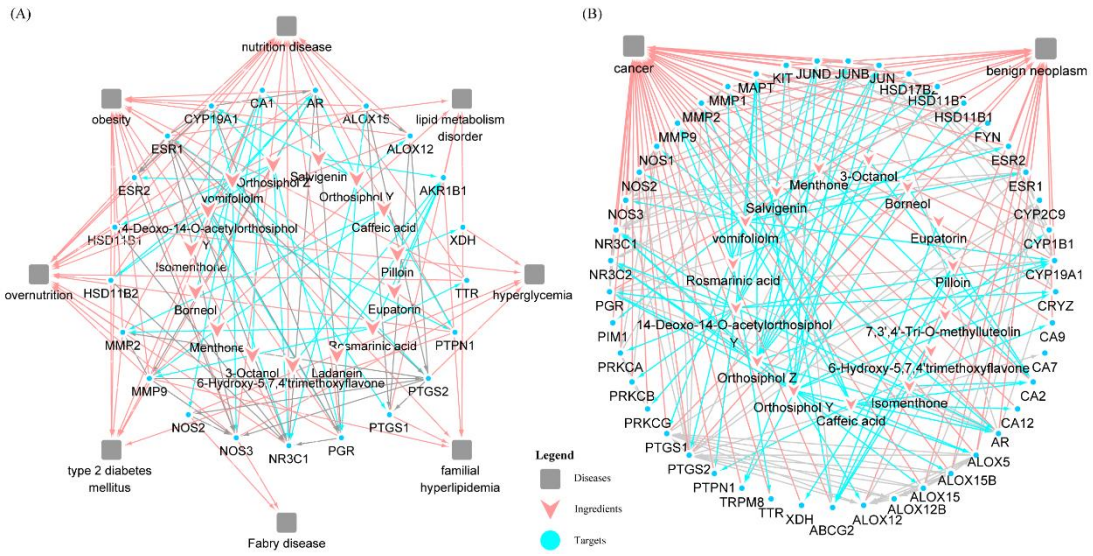

Figure 4 the Ingredient-target-disease (I-T-D) network of *orthosiphon aristatus*.

3.6.2 The molecular basis of Antiproliferative

Orthosiphon stamineus was rarely used for neoplasm in history of folk medicine, but our results shown that 15 active ingredients and 42 target genes concerned with benign neoplasms and cancers (figure 4B). Figure 5 illustrated that Orthosiphol Y, Orthosiphol Z and 14-Deoxo-14-O-acetylorthosiphol Y were the core compounds, and the hub genes such as ALOX12, JUN, JUND, PTGS2, PGR, ABCG2, NR3C1 were all the key molecules of antiproliferative activity. Thus, through regulating of these targets, Orthosiphon stamineus could regulate some signal (i.e., nitrogen metabolism, pathways

in cancer, non-small cell lung cancer, VEGF signaling pathway, ErbB signaling pathway, Wnt signaling pathway, etc.) for antineoplastic effects (the detail information was listed in Table 1).

3.6.3 Anti-inflammatory and anti-infectious property

As can be seen from table 1, 18 target genes and eight constituents had an association with infectious diseases, which suggested that these molecules could play a vital role in treating infection or inflammation. Consideration with the resultant of pathway analysis such as "Leukocyte transendothelial migration," "MAPK signaling pathway," "African trypanosomiasis" above, we believe that eight compounds Ladanein, Salvigenin, Caffeic acid, Orthosiphol Y, Orthosiphol Z, Vomifoliol, Rosmarinic acid, and 14-Deoxo-14-O-acetylorthosiphol Y could regulate essential proteins (i.e., MAPT, PRKCA, PRKCA, PRKCB, JUN, JUND, NOS2, PTGS2, etc.) to promote our body's resistance against the infectious agent and to remain balance of immunity.

3.6.4 Other conditions

Beside the results described above, we have listed 10 diseases classified by anatomy based on the anatomical structure in the DO database. These 10 diseases were totally mapped to 16 potential active ingredients and 64 target genes in my study, and the details could be found in table 1.

Some important indications in the table 1 were shown that *orthosiphon aristatus* could treat cardiovascular disease, gastrointestinal disease, respiratory disease, endocrine disease, urinary system disease, immune system disease, nervous system disease and so on through binding related targets, and some of them shared same targets or same signaling pathways. For instance, MMP9 were shared by almost all categories as well as PTGS, NOS, ESR, NR3C1, JUN, KIT, ALOX15 have higher frequency in these 10 diseases (Figure), and several signaling pathways, such as Arachidonic acid metabolism, VEGF signaling pathway, Calcium signaling, Leukocyte trans-endothelial migration, Aldosterone-regulated sodium reabsorption, Steroid hormone biosynthesis, were likely to be involved in most disorders from our DO analysis.

Table 1 The potential active ingredients from orthosiphon aristatus and their targets, signal pathway and treated diseases

DO Terms	Diseases	Ingredients	Target	Related Signal Pathway
disease of cellular proliferation	benign neoplasm, cancer	vomifoliol, Rosmarinic acid, 14-Deoxo-14-O-acetylorthosiphol Y, Orthosiphol Z, Orthosiphol Y, Caffeic acid, Salvigenin, 6-Hydroxy-5,7,4'trimethoxyflavone, 7,3',4'-Tri-O-methylluteolin, Pilloin, Eupatorin, Borneol, 3-Octanol, Menthone, Isomenthone,	ESR2, MMP2, ESR1, CYP19A1, PTGS2, MMP1, PGR, JUN, LOX15B, AR, MMP9, ALOX5, CA9, ALOX15, HSD11B2, KIT, OS2, PTGS1, ALOX12, HSD11B1, ABCG2, CYP1B1, JUNB, JUND, MAPT, PTPN1, TRPM8, CA12, CA2, CRYZ, FYN, HSD17B2, NR3C2, NR3C1, XDH, PRKCA, PRKCG, TTR, PRKCB, NOS3, NOS1, PIM1	Nitrogen metabolism, Arachidonic acid metabolism, Pathways in cancer, VEGF signaling pathway, Calcium signaling, Bladder cancer, Non-small cell lung cancer, Glioma, Arginine and proline metabolism, ErbB signaling pathway, GnRH signaling pathway, Wnt signaling pathway, Phosphatidylinositol signaling system, Natural killer cell mediated cytotoxicity
disease by infectious agent	parasitic infectious disease, viral infectious disease, bacterial infectious disease	Ladanein, Salvigenin, Caffeic acid, Orthosiphol Y, Orthosiphol Z, 14-Deoxo-14-O-acetylorthosiphol Y, vomifoliol, Rosmarinic acid	ADORA2A, ALOX5, ESR1, JUN, JUND, MMP1, MMP2, MMP9, NOS1, NOS2, NOS3, PTGS1, PTGS2, MAPT, PRKCA, PRKCB	African trypanosomiasis, Leukocyte trans-endothelial migration, Leishmaniasis, Vibrio cholerae infection, Amoebiasis, MAPK signaling pathway
disease of metabolism	acquired metabolic disease, inherited metabolic disorder	6-Hydroxy-5,7,4'trimethoxyflavone, Ladanein, Rosmarinic acid, Eupatorin, Salvigenin, Pilloin, Orthosiphol Y, Orthosiphol Z, 14-Deoxo-14-O-acetylorthosiphol Y, vomifoliol, Caffeic acid, Isomenthone, Borneol, Menthone, 3-Octanol	AKR1B1, ALOX12, ALOX15, AR, CA1, CYP19A1, ESR1, ESR2, HSD11B1, HSD11B2, MMP2, MMP9, NOS2, NOS3, NR3C1, PGR, PTGS1, PTGS2, PTPN1, TTR, XDH	Arachidonic acid metabolism, Aldosterone-regulated sodium reabsorption, Steroid hormone biosynthesis, Arginine and proline metabolism, Linoleic acid metabolism

Table 1 The potential active ingredients from orthosiphon aristatus and their targets, signal pathway and treated diseases

DO Terms	Diseases	Ingredients	Target	Related Signal Pathway
disease of anatomical entity	cardiovascular system disease	Ladanein, Salvigenin, 6-Hydroxy-5,7,4'trimethoxyflavone, Rosmarinic acid, Eupatorin, Pilloin, Orthosiphol Y, Caffeic acid, Orthosiphol Z, 14-Deoxo-14-O-acetylorthosiphol Y, vomifoliol, Borneol, Isomenthone, 3-Octanol, Menthone	ADORA1, ADORA2A, ADORA3, AKR1B1, ALOX12, ALOX15, ALOX5, AR, CA12, CA2, CYP19A1, CYP2C9, ESR1, ESR2, HMGCR, HSD11B2, JUN, KIT, MAPT, MMP1, MMP2, MMP9, NOS1, NOS2, NOS3, NR3C1, NR3C2, PRKCB, PTGS1, PTGS2, PTPN1, TTR, XDH	VEGF signaling pathway, Arachidonic acid metabolism, Aldosterone-regulated sodium reabsorption, Calcium signaling pathway, Leukocyte trans-endothelial migration, Vascular smooth muscle contraction
disease of anatomical entity	gastrointestinal system disease	Caffeic acid, Orthosiphol Y, Orthosiphol Z, 14-Deoxo-14-O-acetylorthosiphol Y, vomifoliol, Borneol, Isomenthone, 3-Octanol, Menthone, Salvigenin, Rosmarinic acid, Eupatorin, 7,3',4'-Tri-O-methylluteolin, 6-Hydroxy-5,7,4'trimethoxyflavone, Pilloin	ALOX5, AR, CA2, ESR1, ESR2, JUN, KIT, MMP1, MMP2, MMP9, NOS1, NOS2, OPRD1, PTGS1, PTGS2, PTPN2, XDH	Gastric acid secretion, Salivary secretion, Pancreatic secretion, Bile secretion
disease of anatomical entity	respiratory system disease	Ladanein, Salvigenin, Orthosiphol Y, 7,3',4'-Tri-O-methylluteolin, Rosmarinic acid, Orthosiphol Z, vomifoliol, 14-Deoxo-14-O-acetylorthosiphol Y, Caffeic acid, 3-Octanol, Borneol, Pilloin	ADORA2A, ALOX15, CYP1B1, FYN, HSD11B2, JUN, MMP1, MMP2, MMP9, NOS1, NOS2, NOS3, NR3C1, PRKCA, PTGS1, PTGS2, TRPM8, TTR, XDH	Leukocyte trans-endothelial migration, Arachidonic acid metabolism, Calcium signaling pathway, Steroid hormone biosynthesis
disease of anatomical entity	endocrine system disease	Orthosiphol Z, 14-Deoxo-14-O-acetylorthosiphol Y, vomifoliol, Orthosiphol Y, Caffeic acid, Rosmarinic acid	AR, CYP19A1, ESR1, ESR2, HMGCR, HSD11B1, HSD11B2, HSD17B2, MMP2, MMP9, NOS3, NR3C1	Steroid hormone biosynthesis, Aldosterone-regulated sodium reabsorption, Linoleic acid metabolism

Table 1 The potential active ingredients from orthosiphon aristatus and their targets, signal pathway and treated diseases

DO Terms	Diseases	Ingredients	Target	Related Signal Pathway
disease of anatomical entity	immune system disease	6-Hydroxy-5,7,4'trimethoxyflavone, 7,3',4'-Tri-O-methylluteolin, Pilloin, Eupatorin, Salvigenin, Orthosiphol Y, Caffeic acid, vomifoliol, Rosmarinic acid, 14-Deoxo-14-O-acetylorthosiphol Y, Orthosiphol Z, 3-Octanol, Borneol	ABCG2, ALOX15, CA3, CYP1B1, ESR1, ESR2, FYN, MMP2, MMP9, NOS1, NOS3, NR3C1, TRPM8, TTR, PRKCA, PRKCG, PRKCB, JUN, JUND, MAPT	Steroid hormone biosynthesis, VEGF signaling pathway, Calcium signaling pathway, Leukocyte trans-endothelial migration, Focal adhesion, Natural killer cell mediated cytotoxicity, Fc epsilon RI signaling pathway, Wnt signaling pathway, MAPK signaling pathway
disease of anatomical entity	urinary system disease	Ladanein, Salvigenin, 6-Hydroxy-5,7,4'trimethoxyflavone, Rosmarinic acid, Eupatorin, Pilloin, Caffeic acid, Isomenthone, Borneol, Menthone, 3-Octanol, Orthosiphol Z, vomifoliol, 14-Deoxo-14-O-acetylorthosiphol Y, Orthosiphol Y	ADORA2A, AKR1B1, CA1, CA2, CYP2C9, ESR1, HSD11B2, JUN, JUND, KIT, MMP1, MMP2, MMP9, NOS1, NOS2, NOS3, NR3C1, NR3C2, PRKCA, PRKCB, PTGS1, PTGS2, XDH	Aldosterone-regulated sodium reabsorption, Steroid hormone biosynthesis, Calcium signaling pathway, Proximal tubule bicarbonate reclamation
disease of anatomical entity	integumentary system disease	Salvigenin, Ladanein, Caffeic acid, Orthosiphol Y, Rosmarinic acid	ADORA3, ALOX5, KIT, MMP9	Arachidonic acid metabolism, Melanogenesis
disease of anatomical entity	musculoskeletal system disease	Ladanein, Salvigenin, Orthosiphol Y, Orthosiphol Z, 14-Deoxo-14-O-acetylorthosiphol Y, vomifoliol, Caffeic acid, Isomenthone, Borneol, Menthone, 3-Octanol, 7,3',4'-Tri-O-methylluteolin, Rosmarinic acid	ADORA1, ALOX15, AR, CA1, CYP19A1, CYP1B1, ESR1, ESR2, HSD11B1, JUN, JUNB, JUND, KIT, MAPT, MBNL1, MMP1, MMP2, MMP9, NOS1, NOS2, NOS3, NR3C1, NR3C2, PRKCA, PRKCG, PRKCB, PTPN1	Vascular smooth muscle contraction, Calcium signaling pathway, Phosphatidylinositol signaling system, Osteoclast differentiation

Table 1 The potential active ingredients from orthosiphon aristatus and their targets, signal pathway and treated diseases

DO Terms	Diseases	Ingredients	Target	Related Signal Pathway
disease of anatomical entity	reproductive system disease	Orthosiphol Y, Orthosiphol Z, 14-Deoxo-14-O-acetylorthosiphol Y, vomifoliol, Caffeic acid, Rosmarinic acid	ALOX15, AR, CYP19A1, ESR1, ESR2, HSD17B2, MMP1, MMP2, MMP9, NOS3, PGR, PRKCA, PRKCG, PRKCB, PTGS2, JUN	GnRH signaling pathway, VEGF signaling pathway, Steroid hormone biosynthesis, Wnt signaling pathway, Calcium signaling pathway
disease of anatomical entity	nervous system disease	6-Hydroxy-5,7,4'trimethoxyflavone, 7,3',4'-Tri-O-methylluteolin, Pilloin, Eupatorin, Salvigenin, Orthosiphol Y, Caffeic acid, Orthosiphol Z, 14-Deoxo-14-O-acetylorthosiphol Y, vomifoliol, 3-Octanol, Borneol, Rosmarinic acid	ABCG2, ALOX12, ALOX15, ALOX5, AR, CA12, CA3, CA4, CYP19A1, CYP1B1, CYP2C9, ESR1, ESR2, FYN, HSD11B1, KIT, MAPT, MMP1, MMP2, MMP9, NOS1, NOS2, NOS3, NR3C1, PGR, PRKCG, PTGS1, PTGS2, TDP1, TTR, XDH	Calcium signaling pathway, Long-term depression, Long-term potentiation, Steroid hormone biosynthesis, Leukocyte trans-endothelial migration

4 DISCUSSION AND CONCLUSION

Orthosiphon aristatus was extensively used for edema, hypertension, diabetes, obesity, rheumatism, nephrolithiasis, urinary inflammation, hyperlipidemia by decocting or making herb tea in our traditional folk medicine[3, 4]. So far, accumulating evidence indicates that it has several pharmacological functions, including diuretic, antioxidant, anti-inflammatory, antimicrobial, anti-proliferative, anti-angiogenesis, hepatoprotective activities and ameliorate metabolism[9]. In this study, we collected 139 compounds and evaluated its by ADMET model, then predicted water solubility, gastrointestinal absorption, blood-brain barrier permeation, drug-likeness (DL) and oral bioavailability (OB), screened potential ingredients and conducted their targets prediction. In the end, we generated several ingredient-target-disease networks to explore potential pharmacological mechanism and extend understanding of *O. aristatus* for drug discovery.

Consideration for good water solubility, high oral availability, and excellent drug-likeness, we screened out 52 potentially active ingredients, including 35 volatile oils (67.31%), 19 terpenoids, 7 flavonoids, and several phenols. In general, volatile oils consist of terpenoids, aliphatic, and aromatic compounds and their oxygen-containing derivatives and could sustain loss during boiling and drying[31]. Moreover, flavonoids, terpenoids, and phenols all have anti-inflammatory, analgesic, anti-microbial, antiproliferative activities, and immune regulation, as do volatile oils. Thus, it indicated that the main active ingredients of *O. aristatus* were volatile oils, terpenoids, flavonoids, and several phenols and suggested us optimizing the processing technology and the usage for avoidance loss of active ingredients.

Results of targets prediction and molecular docking demonstrated that the therapeutic effects of *O. aristatus* were in association with 16 ingredients, and the relationship of ingredient-target interaction was great reliable because most ligand-receptor had a strong binding affinity. Based on the binding free energies of docking, the ingredients orthosiphol Z, orthosiphol Y, and rosmarinic acid all have excellent binding affinity to their target proteins (figure). Till to date, there is not any published paper about the pharmacological activity of orthosiphol Y, Z, and only the orthosiphol A, an isomer of orthosiphol Y, Z, has been proven to benefit diabetes and gout due to that retard intestinal maltase function[32] and promote UA excretion[9]. However, the water solubility of the orthosiphol A is poor, and the gastrointestinal absorption is low in our ADMET model. Different from orthosiphol A, rosmarinic acid, a water-soluble and absorbable polyphenol that been researched endlessly, had several exciting effects, including antioxidant[33], anti-inflammatory[34], anti-tumor[35], antimicrobial[36], anti-diabetic[37], neuroprotective[38, 39] and nephroprotective activities[40, 41] even cosmetic effects[42]. Similarly, two flavonoids Eupatorin and Sinensetin also been confirmed to possess almost the same pharmacological effects as *O. aristatus*[4, 43-45]. Therefore, we speculate that the *O. aristatus* own many ponderable ingredients for phytochemically screening and validating.

GO and KEGG pathway analyses were conducted to better elucidate the pharmacological mechanism of *O. aristatus.*

In past decades, accumulated researches have evaluated the antineoplastic effects of

O. aristatus in vivo and in vitro, and it showed that the standardized extracts of *O. aristatus* could inhibit the growth of hepatocellular carcinoma, fibrosarcoma, and colorectal carcinoma cell line by inducing apoptosis[5, 6, 46] or antimutagenic efficacies[47]. The chloroform extract of *O. aristatus* had solid inhibitory activities against five cancer cell lines, and Luo *et al*.[48] concluded that the backbones of diterpenoids had a significant influence on cell toxicity due to the six active compounds (Spicatusene B, Spicatusene C, Orthosiphol R, Orthosiphol N, Orthoarisin A, Orthosiphonone D). Ahamed *et al*.[6] further studied the antiproliferative activities of the ethanolic extract of O. aristatus in athymic mice with colorectal tumor, and it caused 47.62 ± 6.4% and 83.39 ± 4.1% tumor growth suppression after four weeks at the dose of 100 and 200 mg/kg body weight, respectively. Interestingly, the ethanolic extract of *O. aristatus* could significantly reduce the vascular endothelial growth factor (VEGF) level and suppress the tumorous angiogenesis via the VEGF-VEGFR signal[6], which has been depicted in our results (Figure 3). Another research suggested that the methanolic extract of *O. aristatus* synergistically enhanced cytotoxic effect towards MCF-7 hormone-sensitive human breast cancer cells with Tamoxifen. Still, the extract itself did not show any anticancer efficacy[49]. Here, the synergistic effect of *O. aristatus* was associated with the hormone metabolic process as mentioned above. In Table 1, We have listed some abnormal signal pathways in tumors from our I-T-D network for a comprehensive understanding of the potential anti-tumor mechanisms of O. aristatus. Furthermore, based on our I-T-D network analysis of O. aristatus, those candidate signaling such as “Nitrogen metabolism,” “Pathways in cancer,” “Arachidonic acid metabolism,” “VEGF signaling pathway,” “Non-small cell lung cancer,” “ErbB signaling pathway,” “Wnt signaling pathway,” and even “the Calcium signaling,” will provide a basis for further pharmacological verifications with confirmation of active ingredients.

In addition, concerning the effect of *O. aristatus* on metabolic diseases, three studies[50-52] indicated that the aqueous extract could ameliorate blood glucose level and lipid profile in diabetic rats as well as did work in pregnant diabetic rats. They further investigated the primary mechanisms that indicated *O. aristatus* probably increases the sensitivity of pancreatic beta cells to insulin or (and) peripheral utilization of glucose by the tissues for lowering plasma glucose[50, 53]. Meanwhile, Chen and Xu *et al*.[9, 54] evaluated the function of anti-gout in mice. They showed that ethanolic and ethyl acetate fraction of *O. aristatus* possessed lowering serum uric acid (UA) and relieving pain because of the increasing excretion and the suppressing production of the UA, anti-inflammation, and analgesia. These studies concluded that some active compounds in *O. aristatus* inhibited the xanthine oxidase activity and targeted proximal tubule epithelial cells for regulation reabsorption, and we did find in our research. Amazingly, an investigation demonstrated the *O. aristatus* might benefit the metabolic diseases mainly through regulating the tricarboxylic acid cycle, gluconeogenesis, lipid, and amino acid metabolism by (1)H NMR spectroscopy[55]. Furthermore, this benefit of *O. aristatus* to our metabolism has been turned out to be reliable by a randomized clinical trial[56]. Our research also manifested a tight connection between metabolism disorders and several good water-soluble ingredients

(Figure 4A, Table 1) and suggested that some pharmacological mechanisms were involved. For example, Arachidonic acid metabolism, a long-established lipid metabolism process, plays an extensive pathophysiological role in human health and diseases, aiding in cell proliferation and tissue homeostasis and in inflammatory diseases, cancer, cardiovascular diseases, and metabolism aberration, such as diabetes mellitus, obesity, lipid alteration[57]. So, it is reasonable to conduct a targeted therapy if the Arachidonic acid metabolism has been impacted by *O. aristatus*.
Besides, it seems compelling for *O. aristatus* to treat nephritis[40, 48, 54], calculus[58], urinary tract infection, hypertension[59], liver damage[60], atherosclerosis[61], and gastritis[62] in numerous animal experiments. A few investigations also evaluated its neuroprotection in epilepsy, depression, and Alzheimer's disease[45]. However, most of these studies were at the pre-clinical phase; only three clinical trials of an herbal combination containing *O. aristatus* proved effective in treating hypertension, metabolic syndrome[56], and urinary tract infections[14, 15]. It is necessary for us to carry out more clinical trials and comprehensive pharmacological and biochemical studies to validate the clinical efficacy and active compound(s).
In summary, the results of this integrative analysis provided a comprehensive understanding of the physicochemical property and the likelihood for a natural drug, and clarified the polypharmacological mechanisms via the relationship among active ingredients, targets, and diseases. Nevertheless, the principal limitation of this analysis was the lack of validation of experiments. Conversely, it provided better guidance for subsequent experiments and also provided some novel mechanisms for clinical practice and potential active ingredients for drug discovery. Noteworthily, the reliable systems pharmacology platform of folk medicine should be constructed to develop ethnomedicines.

DATA AVAILABILITY STATEMENT
All available datasets generated of this study are included in the **Supplementary Materials**.

AUTHOR CONTRIBUTIONS
Xingqiang Wang and Dongyun Lei contributed equally to this work

CONFLICTS OF INTEREST
None declared.

FINDING
This work is supported by grants from National Natural Science Foundation of China (81960863, 81960870), the Clinical Trial for the treatment of Rheumatoid Arthritis with Warming yang and Smoothening Meridians (201507001-07, registration number: ChiCTR-INR-16010290), the Construction Project of National Traditional Chinese Medicine Clinical Research Base (2018 No. 131), the "Chinese Medicine Modernization Research" key project of China's National Key R&D Programmes

(2017YFC1704005), the Funding of Yunnan Applied Basic Research Projects-Union Foundation (2019FF002(-082), 2019FF002(-031)), and the Funding of Yunnan Provincial Health Science and Technology Plan (2018NS0045, 2018NS0046).

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