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Research Article

Systematic Pharmacology-Based Strategy to Investigate the Mechanism of *Vladimiriae Radix* in Benign Prostatic Hyperplasia

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Abstract

Objective: The present study aimed to elucidate the mechanism of action of the dried roots of *Vladimiriae Radix* against benign prostatic hyperplasia (BPH) using network pharmacology, molecular docking, and cell-level experimental verification technologies, thereby providing experimental evidence for basic research, clinical application, and the modernization research of Tibetan-Chinese medicine integrated medication. **Methods**: Firstly, the active components of *Vladimiriae Radix* were screened using the TCM Systems Pharmacology Database and Analysis Platform (TCMSP) and PubChem, with the criteria of oral bioavailability (OB) $\geq$30% and drug-likeness (DL) $\geq$0.18. Subsequently, the SwissTargetPrediction database was used to identify potential targets for the components, and an overlap analysis was conducted on the BPH-related targets from GeneCards, Online Mendelian Inheritance in Man (OMIM), and the Therapeutic Target Database (TTD) to identify the common targets. Then, STRING and Cytoscape 3.10.3 analyses were used to construct the protein-protein interaction (PPI) network and the "Chinese medicine-component-target-disease" network for screening core targets. Gene ontology (GO)/kyoto encyclopedia of genes and genomes (KEGG) enrichment analyses were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) database and bioinformatics platforms. Discovery Studio 2019 was used to verify the binding between components and targets, and AutoDockTools1-2 was employed to calculate the binding energy. Cell-level experiments (CCK-8 assay and RT-PCR) were conducted using BPH-1 cells to validate the effect of the representative component oleanolic acid. **Results**: A total of 235 common targets were identified between *Vladimiriae Radix* and BPH, and 6 core targets, including *AR, CYP17A1*, *CYP19A1*, *ACHE*, *F2*, and *HMGCR*, were further screened. These core targets are mainly involved in biological functions such as steroid hormone response, cellular response to nutrient levels, and regulation of membrane potential, and are enriched in BPH-related pathways including lipid and atherosclerosis, cholinergic synapse, and AGE-RAGE. Molecular docking verification found that the active components form stable bindings with the core targets. Cell experiments found that oleanolic acid significantly inhibits BPH-1 cell proliferation and regulates the mRNA expression of the six core targets at concentration of 10 μM, 20 μM, 40 μM (significantly downregulated the mRNA expression of *AR* and *HMGCR* ($p < 0.05$), significantly upregulated the mRNA expression of *CYP17A1*, *CYP19A1*, and *ACHE* ($p < 0.05$), and had no significant effect on *F2*). **Conclusion**: Costunolide, dehydrocostus lactone, luteolin, quercetin, taraxasterol and oleanolic acid are the main bioactive ingredients in *Vladimiriae Radix*. Among them, oleanolic acid exhibited the highest binding energy with 6 core targets and exhibits anti-BPH properties. The present study fills the research gap in the anti-BPH mechanism of *Vladimiriae Radix*, validates the efficacy of the active components in *Vladimiriae Radix* at the cellular level, and provides clear targets and theoretical support for subsequent pharmacological verification, active component development, and clinical translation.

Keywords: *Vladimiriae Radix*; network pharmacology; molecular docking; benign prostate hyperplasia; pharmacological action

1. Introduction

Benign prostatic hyperplasia (BPH) is a common benign disease among elderly men [1]. Its pathological features include the proliferation of epithelium, fibromuscular tissue, glands, and stromal cells in the transition zone of the prostate and the perurethral area, which in turn leads to benign enlargement of the prostate. This condition is often accompanied by bladder outlet obstruction and clinically manifests as lower urinary tract symptoms (LUTS) [2]. The combination of these two is collectively referred to as BPH/LUTS. Specifically, LUTS includes frequent urination, urgent urination, urinary incontinence, increased nocturia, delayed urination, poor urine flow, and a feeling of incomplete bladder emptying [3]. These symptoms not only significantly reduce patients' quality of life but also easily trigger negative emotions, impair physical and mental health, and affect family and social harmony. From an epidemiological perspective, the incidence of BPH shows a significant positive correlation with age. The incidence rate is approximately 50% among men over 50 years old, reaches 83% among those over 80 years old, and about 90% of men over 90 years old have varying degrees of prostatic hyperplasia. the incidence rate among men over 40 years old is around 8% [4]. With the intensification of population aging, clinical attention to this condition has continued to increase. Currently, clinical treatment mainly relies

on surgery and medications. The first-line pharmacotherapeutic regimen for BPH/LUTS involves the use of alpha-blockers, 5-alpha-reductase inhibitors, either alone or in combination [5]. However, these medications are not effective for all BPH/LUTS patients, they also tend to cause adverse reactions such as sexual desire disorders and insomnia, and some patients experience drug tolerance issues. These factors limit their clinical application, making it of great significance to explore safe and low-toxicity treatment regimens. In the system of traditional chinese medicine (TCM), BPH is classified under the category of "Long Bi" (dribbling and retention of urine). Its core pathogenesis lies in the dysfunction of bladder qi transformation, with the main disease locations in the kidney and bladder. Syndrome differentiation revolves around kidney deficiency, dampness-heat, and blood stasis [6]. TCM treatment includes internal therapy (oral administration of herbal medicines) and external therapy (such as drug enema, acupuncture, and acupoint application) [7]. It has the advantages of treating both the root cause and symptoms, significant therapeutic effects, and few adverse reactions. TCM treatment not only improves LUTS symptoms in patients but also reduces blood stasis and edema in prostate tissue, thereby assisting in reducing prostate volume [8], which provides an important supplementary approach for BPH treatment.

Vladimiriae Radix is a Tibetan medicine with ethnic characteristics, and it is also a cross-used medicinal species in TCM and Tibetan medicine [9]. Its main producing areas are concentrated in Aba Tibetan autonomous prefecture, Liangshan Yi autonomous prefecture, Xichang, Baoxing, Ya'an (all in Sichuan Province, China), as well as Yunyang, Kaixian, and Nanchuan (all in Chongqing Municipality, China). Its traditional indications align closely with the therapeutic needs of BPH. In Chinese medicine the herb is classified as a qi-regulating drug (pungent, bitter, warm) that promotes flow, assists bladder qi transformation, dispels blood stasis to reduce glandular swelling, and warms to transform damp turbidity in the urethra—directly addressing the "long-bi" (urinary retention) mechanism of BPH. Formulas containing it, such as "Xiangsha Liu-jun-zi Tang", have been shown to relieve accompanying symptoms [10]. In Tibetan medicine it is known as "Ma-nu"; it harmonizes the nyes-pa, opens the urethra, disperses the "flesh nodule" of the prostate, and is commonly incorporated into preparations like "Wu-wei Muxiang San". Contemporary clinical studies found that Chinese- and Tibetan-compound prescriptions containing *Vladimiriae Radix* (e.g., Mu-xiang Dan-shen Yin combined with tamsulosin) can significantly improve IPSS scores, residual urine volume, and maximum urinary flow rate, reduce prostate volume, and cause fewer adverse effects—thereby providing traditional theoretical support for the present study and validating the herb's candidacy for BPH therapy. The *Vladimiriae Radix* herb is cylindrical or semi-cylindrical with longitudinal grooves, slightly curved, measuring 10–30 cm in length and 1–3 cm in diameter. Its surface is yellowish-brown or dark brown, with longitudinal wrinkles; where the outer skin peels off, reticulate fine vascular bundles (resembling a loofah sponge) are visible, and the root head occasionally has an "oil head" (a black, sticky gelatinous substance). The herb is light in weight, hard and brittle, and easy to break, the fracture surface is yellowish-white or yellow, containing sparse dark yellow oil spots and cracks (Fig. 1A,B). The xylem is broad with radial textures, and the center of some samples is withered. It has a slight aroma, a bitter taste, and feels sticky when chewed [11]. Modern research has shown that costunolide and dehydrocostus lactone are the core active components in *Vladimiriae Radix*. Both can significantly inhibit the proliferation of six types of human-derived tumor cells, and the exocyclic double bond at the Δ11(13) position has been initially found as the main active site of sesquiterpenoid compounds for antitumor activity [12]. Specifically, Bocca *et al*. [13] showed that costunolide can interact with microtubules to inhibit the proliferation of human breast cancer MCF-7 cells in a dose-dependent manner (with a significant effect at 100 nmol/L) and microtubules may be its new intracellular target. It was reported that costunolide inhibits the proliferation and induces the apoptosis of MCF-7 cells by regulating the expression of apoptosis-related proteins such as Bax, Bcl-2, p53 and Caspase-3 [14]. On the other hand, Roy and Manikkam [15] found that dehydrocostus lactone can inhibit the activity of thioredoxin reductase 1 (TrxR1) in Henrietta Lacks (HeLa) cells with a half-maximal inhibitory concentration (IC_{50}) of 12.00 µmol/L, triggering the accumulation of reactive oxygen species (ROS) and the collapse of redox homeostasis, which ultimately induces cell apoptosis. Therefore, in-depth exploration of the material basis and mechanism of action of *Vladimiriae Radix* in anti-BPH can provide theoretical support for its rational clinical application and the research and development of related drugs, and thus holds important research significance.

2. Materials and Methods

2.1 Screening of Plant-Related Targets of Vladimiriae Radix

For the screening of active components of *Vladimiriae Radix*, "*Vladimiriae Radix*" was used as the search term to log into the TCM Systems Pharmacology Database and Analysis Platform (TCMSP, https://www.tcmsp-e.com/#/home). The screening criteria were set as oral bioavailability (OB) $\geq$30%, Drug-likeness (DL) $\geq$0.18 to obtain the potential active components of *Vladimiriae Radix*. In addition, combined the reported anti-BPH excellent active components in the references. The screening results were supplemented and improved by combining published research findings to enhance the comprehensiveness and accuracy of the active component

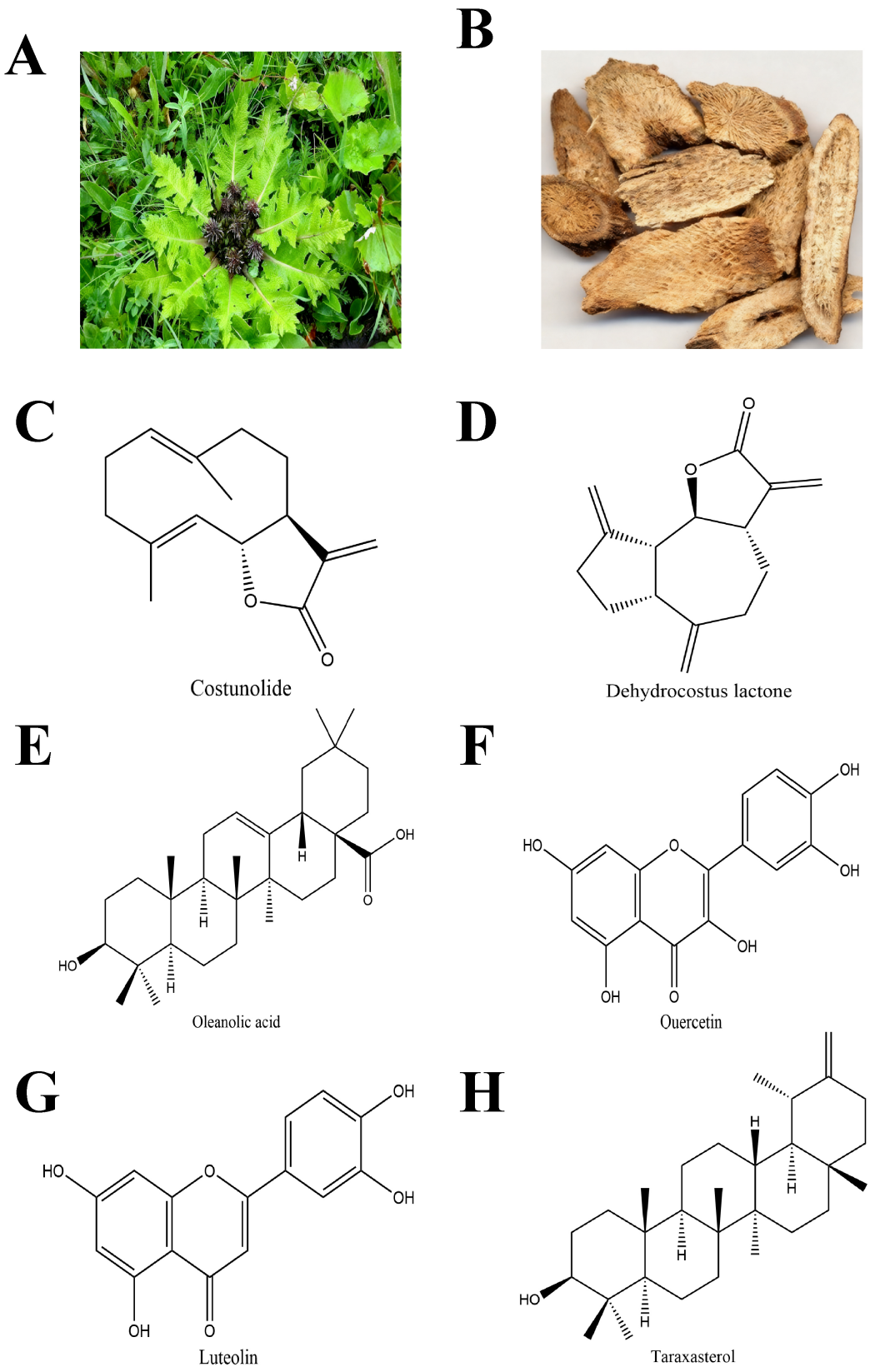

Fig. 1. Original plant, dried medicinal material and main active components of *Vladimiriae Radix*. (A) The original plant of *Vladimiriae Radix* (the plant with rhizomes, roots and leaves). (B) Dried medicinal material of *Vladimiriae Radix*, Core active components which mainly including (C) costunolide, (D) dehydrocostus lactone, (E) oleanolic acid, (F) quercetin, (G) luteolin, and (H) taraxasterol from *Vladimiriae Radix.*

Table 1. Database and software which used in the present study.

Name of database and software	Website address
TCM Systems Pharmacology Database and Analysis Platform (TCMSP)	https://www.tcmsp-e.com/#/database/
PubChem Database	https://pubchem.ncbi.nlm.nih.gov
SwissTarget Prediction Database	https://www.swisstargetprediction.ch/
GeneCards Database	https://www.genecards.org/
Online Mendelian Inheritance in Man (OMIM)	https://omim.org/
Therapeutic Target Database (TTD)	https://db.idrblab.net/ttd/
Venny 2.1 Online Software	https://bioinfogp.cnb.csic.es/tools/venny/index.html
STRING 12.0 Online Database	https://string-db.org/
DAVID 6.8 Database	https://davidbioinformatics.nih.gov/
Microbiological Bioinformatics Platform	https://www.bioinformatics.com.cn/
Lianchuan BioCloud	https://www.omicstudio.cn/tool
PDB Database	https://www.rcsb.org/?ref=nav_home

information. Acquisition of molecular structures of active components and target prediction. Firstly, log into the National Library of Medicine Database (PubChem, https://pubchem.ncbi.nlm.nih.gov) to search for the molecular structures corresponding to the active components obtained from the aforementioned screening. Then, import the molecular structures into the Bioinformatics Analysis Platform for Active Molecule Mechanisms (SwissTargetPrediction, https://www.swisstargetprediction.ch/). Through the target structure prediction function, the potential targets of the active components of *Vladimiriae Radix* were acquired. The names of the websites used for data analysis are provided in Table 1.

2.2 Acquisition of Overlapping Targets Between the Plant and the Disease

Using "BPH" as the search term, search for BPH-related targets in the GeneCards database (GeneCards, https://www.genecards.org/), Online Mendelian Inheritance in Man database (OMIM, https://omim.org/), and Therapeutic Target Database (TTD, https://db.idrblab.net/ttd/) respectively, so as to ensure the acquisition of sufficient target information. Using the VENNY 2.1 plotting platform (https://bioinfogp.cnb.csic.es/tools/venny/index.html), a Venn diagram of the targets related to *Vladimiriae Radix* and the targets related to BPH was generated. By taking the intersection of the two sets of targets, the common therapeutic targets were screened out, thereby clarifying the target association between *Vladimiriae Radix* and BPH.

2.3 Construction of the "Drug Component-Disease-Target" Network and Screening of Core Targets

The disease-drug overlapping targets obtained *via* the Venn diagram and the core targets were imported together into Cytoscape 3.10.3 (Cytoscape Consortium, Seattle, WA, USA) to construct a "drug active component-common target" network. The data were then analyzed to screen proteins based on degree values, followed by visualization of the network.

2.4 Construction of the Protein-Protein Interaction (PPI) Network

To further investigate and visualize the interactions among overlapping targets, first upload the overlapping targets to the search tool for the retrieval of interacting genes/proteins (STRING) database (STRING, https://string-db.org/), set the organism as "Homo sapiens" to acquire data. Then import the obtained data into Cytoscape 3.10.3 software for PPI network analysis and visualization.

2.5 Gene Ontology (GO) Enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Analyses

The overlapping targets were processed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) database (https://davidbioinformatics.nih.gov/): set "official gene symbol" as the select identifier, "gene list" as the list type, and "Homo sapiens" as the organism. With $p < 0.05$ as the threshold, GO functional analysis (including biological process [BP], molecular function [MF] (it is the core quantitative index that measures the "enrichment degree" of the intersection targets (235 in total) between *Vladimiriae Radix* and BPH in a given functional term or pathway, and is used to assess the strength of association between that term/pathway and the anti-BPH effect of *Vladimiriae Radix*), and cellular component [CC]) and KEGG pathway analysis were conducted simultaneously. The obtained overlapping target data were saved and then imported into the Bioinformatics Platform (https://www.bioinformatics.com.cn/) and Lianchuan Bioinformatics Cloud Platform (https://www.omicstudio.cn/tool) for result process-

ing, followed by the generation of GO enrichment bubble plots and heatmaps.

2.6 Molecular Docking

To verify the interaction between the active components of Vladimiriae Radix and the overlapping targets of BPH screened via network pharmacology, the 3D structures (in SDF format) of the main active components of Vladimiriae Radix were downloaded from the PubChem database. Meanwhile, the 3D structures (in PDB format) of the overlapping targets were obtained from the RCSB (Research Collaboratory for Structural Bioinformatics) Protein Data Bank (PDB, https://www.rcsb.org/?ref=nav_home). The above-mentioned component and target structures were uploaded to Discovery Studio 2019 software for docking. Based on the energy values of the binding conformations between ligands and receptor proteins, the docking conformation with the lowest energy and the most stable structure was screened. Furthermore, PyMOL 3.0.3 software (Schrödinger, Inc., New York, NY, USA) was used for visual analysis and processing of the optimal docking results to intuitively present the interaction relationship between the two (the components and targets). Meanwhile, AutoDockTools1-2 (The Scripps Research Institute, La Jolla, CA, USA) was employed to perform a series of operations (such as water removal and hydrogen addition) on the macromolecular protein crystal structures, and finally the binding energy of each component was obtained.

2.7 Cell-Level Experimental Verification

2.7.1 Test Validation

Among the 6 core active components obtained from the network pharmacology analysis of *Vladimiriae Radix*, oleanolic acid was selected as the representative component for this cellular verification, for the following reasons: Molecular docking results showed that oleanolic acid has good binding ability to all key targets of BPH (docking energy $\leq$–7.0 kcal/mol). In addition, oleanolic acid has a stable content in *Vladimiriae Radix* (approximately 0.9%) and commercially available reference standards are available, which facilitates dose control and repeated experiments.

2.7.2 Cell Culture

BPH-1 cells were purchased from Shanghai Jinyuan Biotechnology Co., Ltd. and cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) in a constant-temperature incubator at 37 °C with 5% CO_2. When the cells reached the logarithmic growth phase, they were digested with 0.25% trypsin for passage. Experiments were performed when the cells were passaged to the 3rd generation. BPH-1 cells were validated by short tandem repeat (STR) profiling and tested negative for mycoplasma.

2.7.3 Cell Resuscitation

Frozen BPH-1 cells were retrieved and rapidly thawed in a 37 °C water bath. After centrifugation to remove the cryopreservation medium, the cells were resuspended in DMEM medium containing 20% FBS and double antibodies (penicillin/streptomycin). The cells were seeded into culture dishes and incubated in a 37 °C, 5% CO_2 incubator. When the confluence of BPH-1 cells reached 80%–90%, the culture medium was discarded, and 3 mL of PBS was added to remove dead cells and excess medium, followed by 3 rounds of PBS washing.

2.7.4 Model Establishment

Dihydrotestosterone (DHT) powder was dissolved in anhydrous ethanol to prepare a 10^{-3} M stock solution, which was aliquoted and stored at –20 °C in the dark. During the experiment, the stock solution was diluted to the target concentration of 10^{-7} M with serum-free DMEM medium, ensuring the final ethanol concentration was $\leq$0.1%. Logarithmic-phase BPH-1 cells were digested, seeded into 96-well plates or 6-well plates at an appropriate density, and incubated for 24 h to allow cell adhesion. The old medium was aspirated, and the corresponding medium was added according to the following groups for further culture of 24~72 h: the DHT model group was cultured in DMEM medium supplemented with 500 nM DHT.

2.7.5 Cell Proliferation Inhibition Assay

Logarithmic-phase BPH-1 cells were digested and resuspended in medium to form a single-cell suspension with a concentration adjusted to 1×10^4–5×10^4 cells/mL. 100 µL of the cell suspension was added to each well of a 96-well plate, and sterile PBS was added to the edge wells. The plate was incubated in a 37 °C, 5% CO_2 incubator for 24 h to allow cell adhesion. The old medium in the wells was aspirated, and medium containing different concentrations of drugs was added according to the experimental design (100 µL per well). After further culture for 24 h, cell morphology was observed under an inverted fluorescence microscope.

Six groups were set up in the experiment: blank control group (medium without drugs), model group (500 nM DHT), positive drug group (500 nM DHT + 40 µM finasteride), low-concentration drug group (500 nM DHT + 10 µM oleanolic acid), medium-concentration drug group (500 nM DHT + 20 µM oleanolic acid), and high-concentration drug group (500 nM DHT + 40 µM oleanolic acid). Each group had at least 3 replicate wells. The plate was returned to the incubator for continuous culture of 48 h. 10 µL of CCK-8 reagent was added to each well, and after gently shaking the 96-well plate, it was incubated in the incubator for another 1–2 h. The absorbance value (OD value) of each well at 450 nm wavelength was detected using a microplate reader. The cell proliferation inhibition rate (R) was calculated according to Formula (1): $R = (1 - OD_{experiment} / OD_{control}) \times 100\%$.

Table 2. Primers used for qRT-PCR.

Target genes	Primer sense (5′-3′)	Primer antisense (5′-3′)
ACHE	CTTCTCCTCCTCCTCTGGCT	ATGCCCAGGAAAGCAGAGAC
AR	ACACCAAAGGGCTAGAAGGC	GTAGTCGCGACTCTGGTACG
CYP17A1	TCCTGCTGCACAATCCTCAG	ATAGTTGGTGTGCGGCTGAA
CYP19A1	TGCGAGTCTGGATCTCTGGA	AGTTTGCTGCCGAATCGAGA
F2	AGATGGGCTGGATGAGGACT	AGCCAAAGGTCCTCGGATTG
HMGCR	TGTGTGTGGGACCGTAATGG	GCAAGCTCCTTGGAGGTCTT

2.7.6 RT-PCR Verification of mRNA Expression of Signaling Pathway-Related Genes

BPH-1 cells were lysed in 1 mL of Trizol, and total RNA was extracted according to the Trizol kit instructions. cDNA templates were synthesized by reverse transcription using a quantitative real-time PCR kit, and RT-PCR experiments were performed on an ABI7500 real-time PCR system. The reaction conditions were as follows: predenaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 20 s, and extension at 72 °C for 34 s. After the reaction, the mRNA expression levels of 6 core proteins (*AR*, *CYP17A1*, *CYP19A1*, *ACHE*, *F2*, and *HMGCR*) were analyzed respectively. The specific primer components for qRT-PCR are listed in Table 2.

2.8 Statistical Analysis

All data were statistically analyzed using SPSS 21.0 software (IBM Corp., Chicago, IL, USA). The results were expressed as mean ± standard deviation ($\bar{x} \pm s$). One-way analysis of variance (ANOVA) was used for comparisons among multiple groups, and $p < 0.05$ was considered statistically significant

3. Results

3.1 Screening Results of the Targets for Active Components of Vladimiriae Radix

A total of 6 active components were obtained through multi-source data collection and collation (Table 3). The active components of *Vladimiriae Radix* were screened based on the parameters of molecular weight (MW), OB, and drug likeness (DL). These 6 active components included costunolide (Fig. 1C), dehydrocostus lactone (Fig. 1D), oleanolic acid (Fig. 1E), quercetin (Fig. 1F), luteolin (Fig. 1G), and taraxasterol (Fig. 1H) respectively. The six core targets—*AR*, *CYP17A1*, *CYP19A1*, *ACHE*, *F2*, and *HMGCR* (*HMGCR* is the rate-limiting enzyme of cholesterol synthesis; its aberrantly elevated activity leads to cholesterol accumulation in prostatic tissue, which provides excess precursors for androgen synthesis [cholesterol→pregnenolone→testosterone→DHT], aggravating hormonal imbalance, and induces oxidative stress in prostate cells, promotes epithelial–mesenchymal transition (EMT), and drives glandular hyperplasia [16]). After further processing of the active components, 800 potential target-related pieces of information for the active components of *Vladimiriae Radix* were predicted. Following further merging and removal of duplicates, a total of 342 targets of *Vladimiriae Radix* were obtained.

Table 3. Active components of *Vladimiriae Radix*.

Mol ID	Molecule Name	OB (%)	DL	MW
MOL010825	Costunolide	29.07	0.11	232.35
MOL001298	Dehydrocostus lactone	58.57	0.14	230.33
MOL000263	Oleanolic acid	29.02	0.76	456.78
MOL000006	Luteolin	36.16	0.25	286.25
MOL000098	Quercetin	46.43	0.28	302.25
MOL004085	Taraxasterol	8.19	0.74	468.84

OB, Oral bioavailability; DL, drug-likeness; MW, molecular weight.

3.2 Collection of BPH Targets and Prediction of Potential the Rapeutic Targets of Vladimiriae Radix for BPH Treatment

Using “BPH” as the keyword, search for prostate cancer-related targets in the GeneCards database and OMIM database respectively re-extracted from GeneCards using a relevance score threshold >20, eliminating low-relevance genes. A total of 235 intersecting targets were retained. After summarizing and removing duplicates, a total of 5256 BPH-related targets were obtained. The active targets of *Vladimiriae Radix* (Fig. 2A,B) screened in section 3.1 and the disease-related disease targets obtained in this section were jointly imported into the VENNY 2.1 plotting tool for intersection analysis and visual mapping. Finally, 235 potential therapeutic targets of *Vladimiriae Radix* for BPH were obtained, and a Venn diagram was drawn based on this (Fig. 2A).

3.3 Construction of the “Drug-Active Component-Disease-Target” Interaction Network (PPI) Network

To more intuitively present the targets of the active components of *Vladimiriae Radix* in BPH, as well as the interactions between their common targets, this study imported the data into Cytoscape 3.10.3 software to construct a “drug-active component-disease-target” interaction

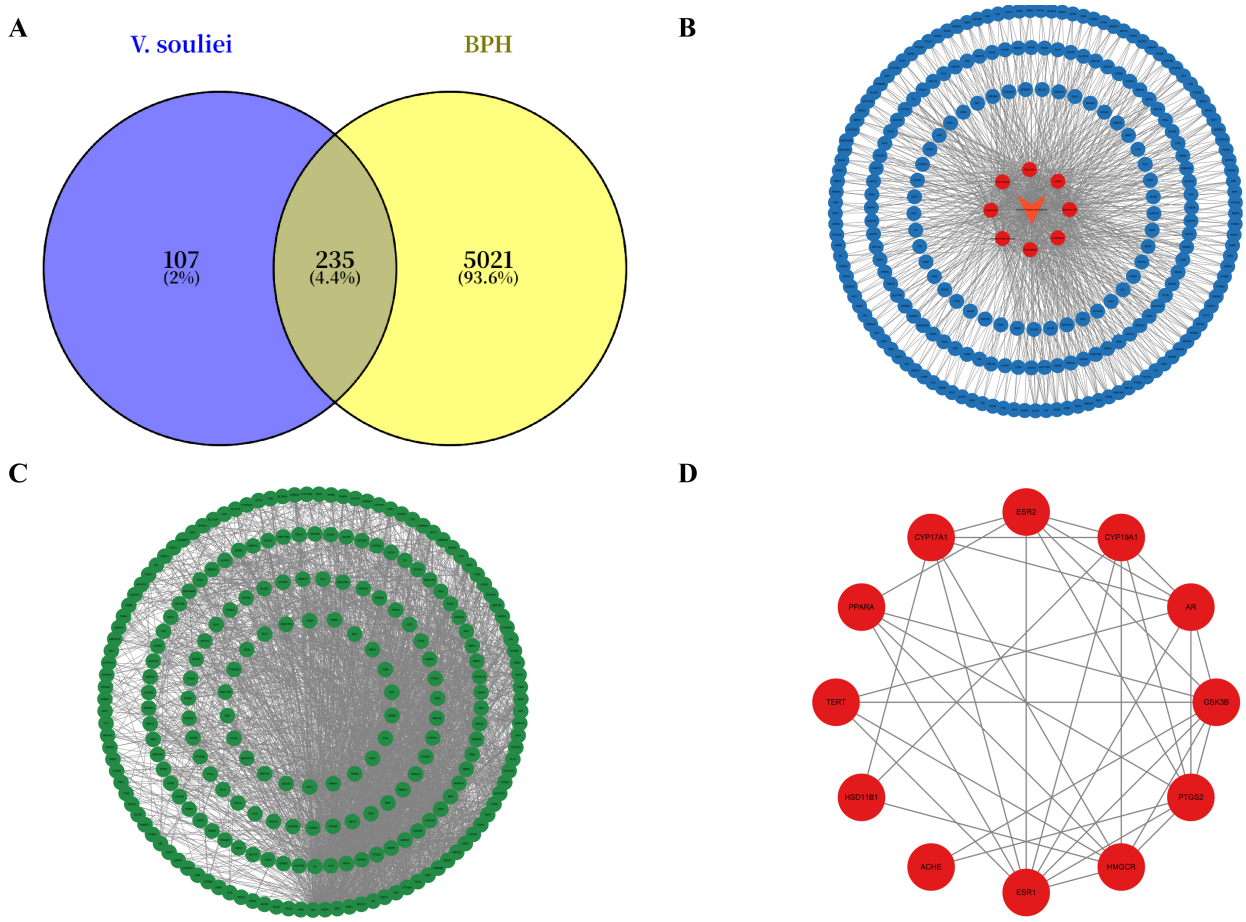

Fig. 2. Common targets, interaction network, core targets and PPI network of *Vladimiriae Radix* against BPH. (A) Venn diagram of common targets between *Vladimiriae Radix* and BPH. (B) Interaction network of targets between *Vladimiriae Radix* and BPH. (C) Fifteen core targets of *Vladimiriae Radix* in treatment of BPH. (D) Protein PPI network diagram. BPH, benign prostatic hyperplasia; PPI, protein-protein interaction.

network diagram and complete the visualization process (Fig. 2B). Among these, 247 protein-protein intersection nodes and 917 interaction edges between target proteins were obtained. The degree of connectivity between targets reflects their importance, with higher connectivity indicating greater importance. Additionally, the top 15 core targets with the highest degree values were screened out using the CytoHubba plugin (Fig. 2C).

3.4 Construction and Visualization of PPI Network

The intersection targets of *Vladimiriae Radix* and BPH were uploaded to the STRING 12.0 database. The species was set to "Homo sapiens" (human), and the minimum interaction score was set to "highest confidence (>0.9)". After removing isolated nodes and performing calculations, a protein-level visualized PPI network was obtained. The network data were then imported into Cytoscape 3.10.3 software for further visualization (Fig. 2D). The importance of targets was reflected by the size of nodes, the depth of node colors, and the density of edges. The network contained 234 nodes and 2877 edges.

3.5 GO Biological Function Analysis and KEGG Pathway Enrichment Analysis

GO functional enrichment analysis was performed on the intersection targets of *Vladimiriae Radix* and BPH using the DAVID database, yielding a total of 678 terms related to BP, 72 terms related to CC, and 225 terms related to MF. The top 10 terms with the highest enrichment significance in each of the three categories were selected and imported into the Online MicroBioInfo Analysis Platform for visualization (Fig. 3A). The specific results are as follows: For BP: The core processes include cellular response to nutrient levels, response to drugs, transport of organic hydroxyl compounds, response to steroid hormones, regulation of membrane potential, response to metal ions, positive regulation of gene expression, response to exogenous stimuli, response to hypoxia, and positive regulation of miRNA transcription. These findings suggest that *Vladimiriae Radix* may exert therapeutic effects by regulating cellular physiological responses through multiple dimensions. For CC: The targets are mainly concentrated in membrane-related structures such as membrane rafts,

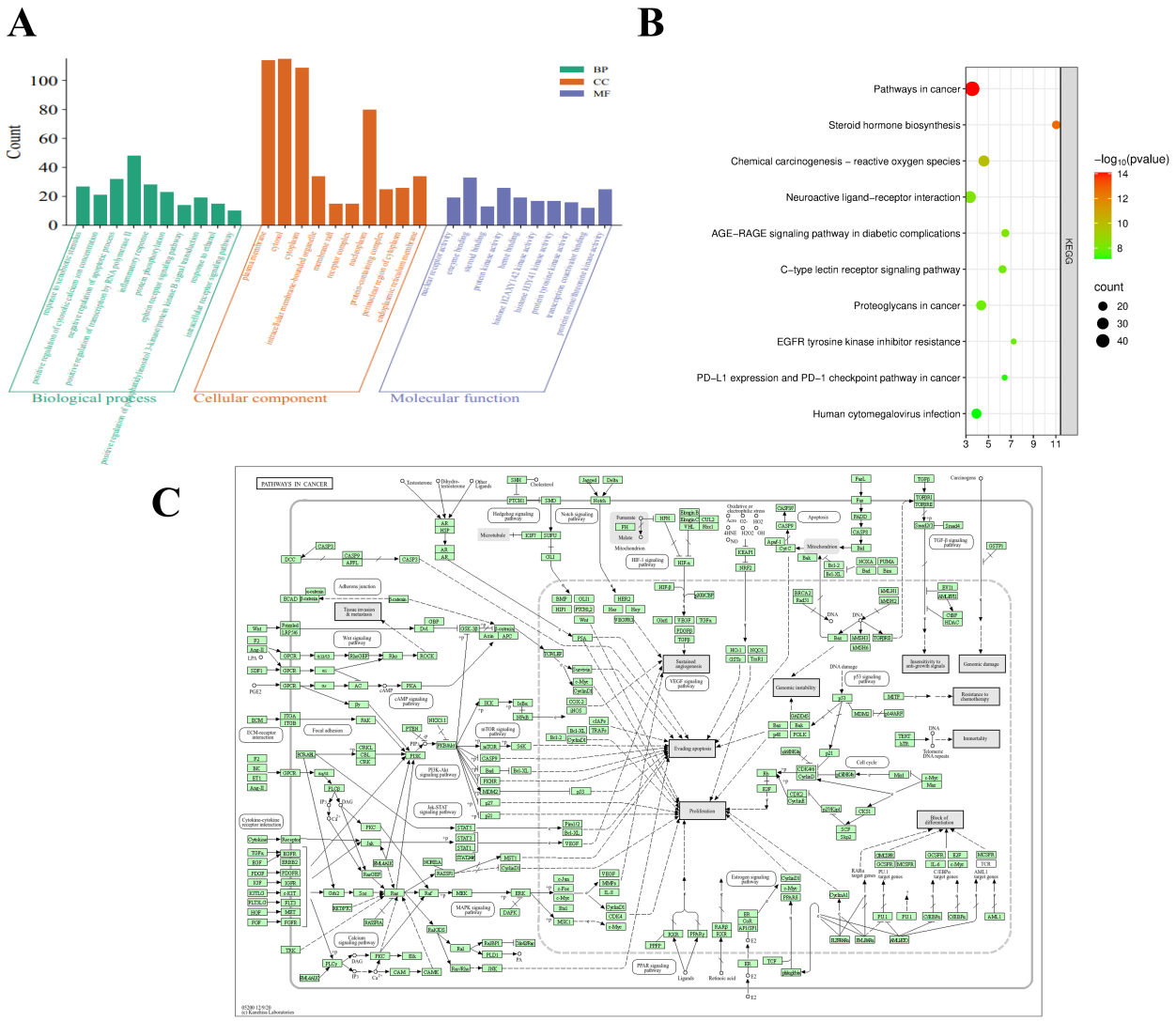

Fig. 3. GO enrichment, KEGG pathway enrichment and cancer-related pathway of core targets. (A) GO Enrichment analysis of key targets of *Vladimiriae Radix.* (B) KEGG enrichment pathways of genes. (C) Pathways in cancer. GO, Gene ontology; KEGG, kyoto encyclopedia of genes and genomes.

membrane microdomains, and membrane regions, and also cover the extracellular space, extracellular region, and cell surface, which reflects the spatial distribution characteristics of the ta3rgets' actions. For MF: The functions are dominated by amide binding, drug binding, phosphatase binding, and G protein-coupled amine receptor activity, and also involve enzyme binding, identical protein binding, and steroid binding, which demonstrates the mechanism of the targets' actions at the molecular level. The results obtained from the GO analysis were further imported into the Online MicroBioInfo Analysis Platform for KEGG pathway enrichment visualization (Fig. 3B). The results showed that the core pathway of *Vladimiriae Radix* in treating BPH was dominated by "Pathways in cancer", and this pathway as well as other related pathways contained multiple important targets associated with the pathological mechanism of BPH (Fig. 3C).

3.6 Molecular Docking

3.6.1 Acquisition of 3D Structures, Molecular Docking, and Visualization Analysis of Active Components and Core Targets of Vladimiriae Radix

Download the SDF-format 3D structure diagrams of the main active components of Vladimiriae Radix from PubChem, obtain the PDB-format 3D structure diagrams of intersection targets from the PDB (Protein Data Bank), upload them to Discovery Studio 2019 for docking, screen the optimal conformations based on the binding conformation energy values of ligands and receptor proteins, and then use PyMOL to conduct visual analysis of the interaction between the two.

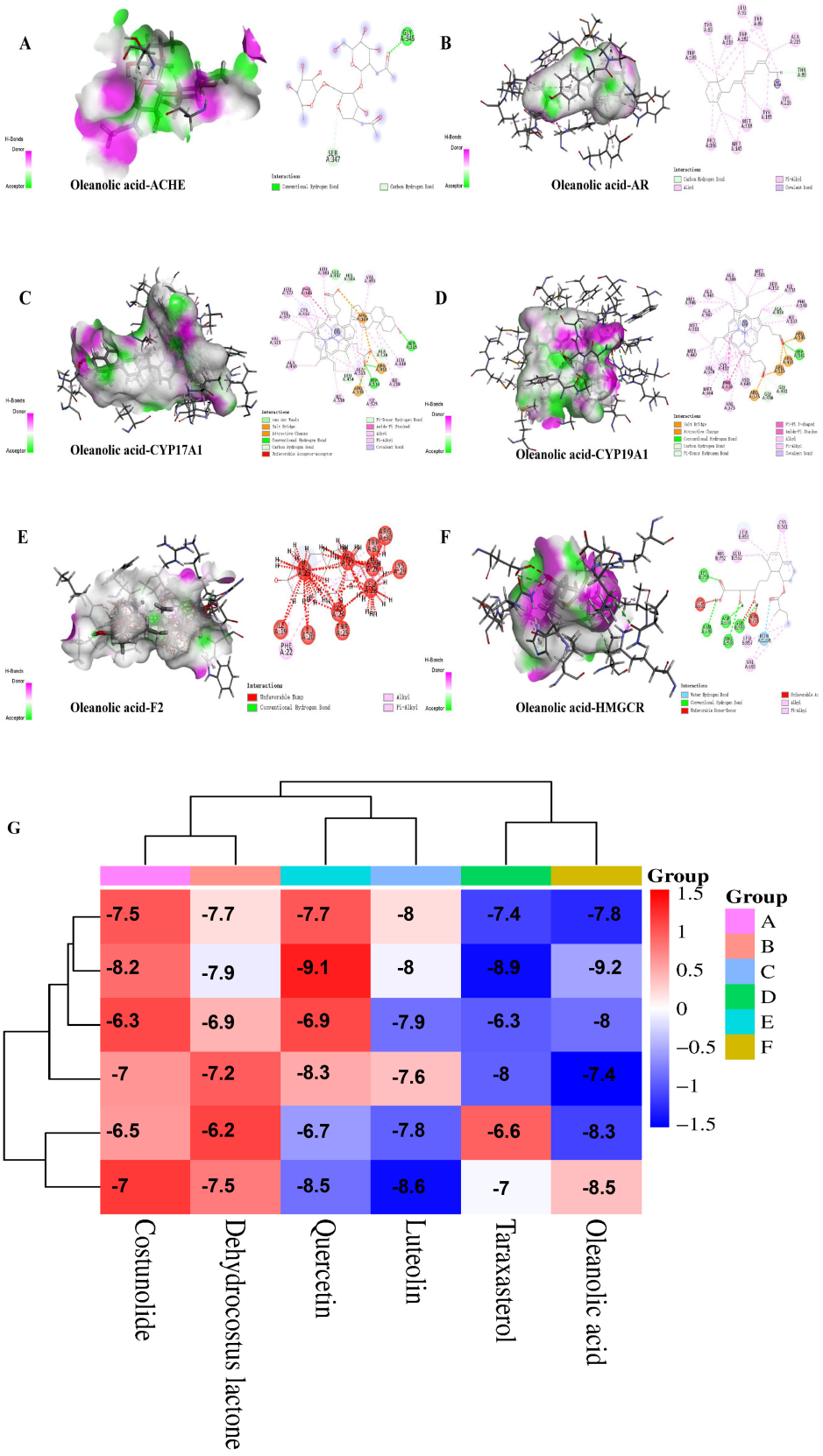

Fig. 4. Interaction of oleanolic acid with core targets and docking energy heatmap. Interaction between oleanolic acid and protein targets (A) *ACHE*, (B) *AR*, (C) *CYP17A1*, (D) *CYP19A1*, (E) *F2*, (F) *HMGCR*. (G) Docking energy heatmap.

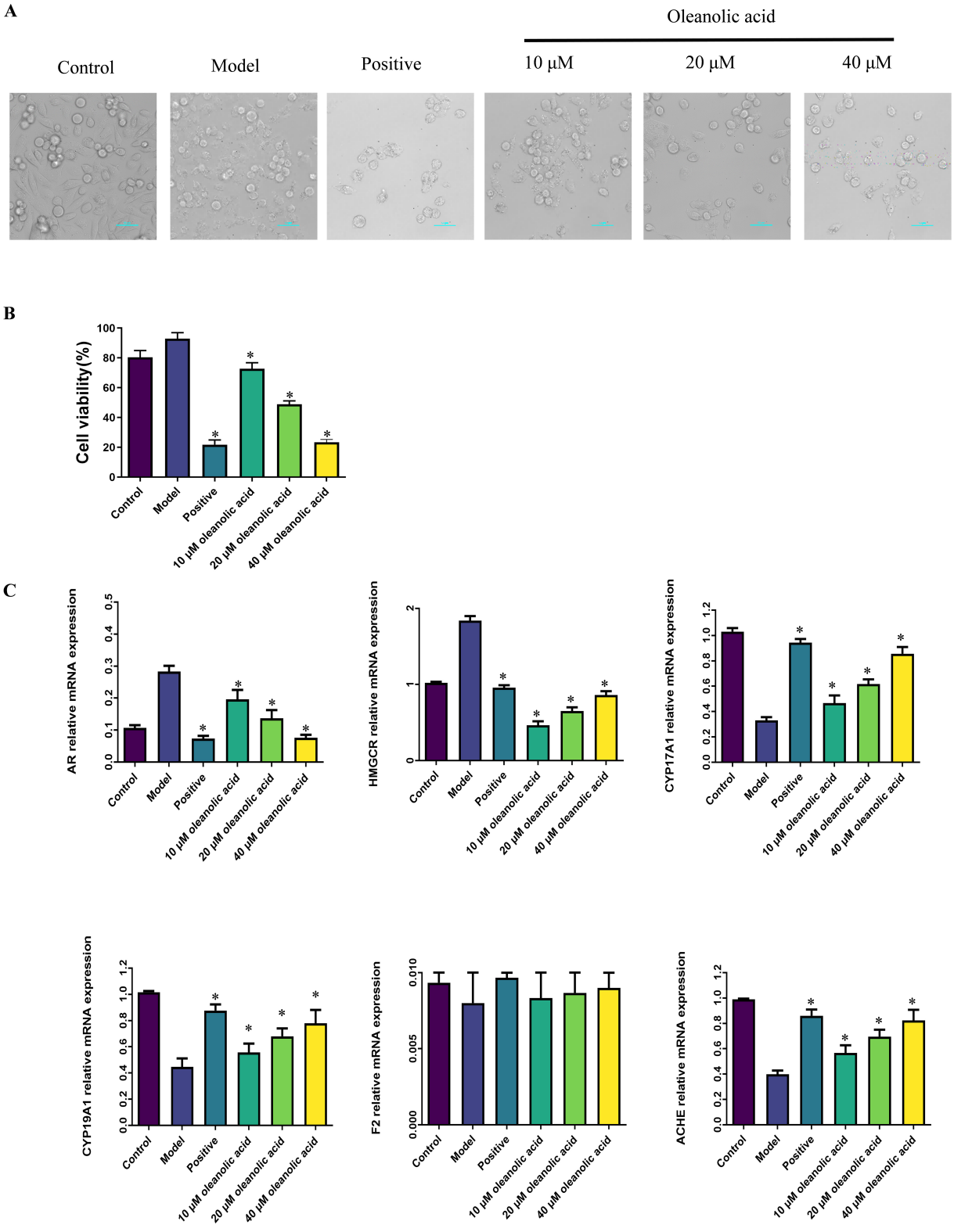

Fig. 5. Effect of oleanolic acid on BPH-1 cell viability and core gene mRNA expression. (A,B) Effect of oleanolic acid on cell viability. Cells were treated with varies concentrations of oleanolic acid for 48 h. Scale bar = 50 μm. (C) Effect of oleanolic acid on *AR*, *HMGCR*, *CYP17A1*, *CYP19A1*, *F2* and *ACHE* mRNA levels in BPH-1 cells. Data were presented as the mean ± SD values performed in triplicate (*$p < 0.05$ *vs*. Model group).

3.6.2 Interaction Between Oleanolic Acid and Protein Targets

The potential binding sites of oleanolic acid and *ACHE* include amino acid residues such as CLY-345 and SER-347, and the compound forms bonds with amino acids like carbon hydrogen bonds and conventional hydrogen bonds (Fig. 4A). The potential binding sites of oleanolic acid and AR include amino acid residues such as LEU-93, ILE-119, TRP-189, TRP-182, MET-118, and TYR-185, and the compound forms bonds with amino acids including carbon hydrogen bonds, pi-alkyl bonds, alkyl bonds, and covalent bonds (Fig. 4B). The potential binding sites of oleanolic acid and *CYP17A1* include amino acid residues such as PHE-446, ARG-109, ARG-138, ARG-451, CYS-453, and ALA-459, and the compound forms interactions with amino acids such as van der waals forces, pi-donor hydrogen bonds, salt bridges, attractive charges, amide-pi stacked bonds, conventional hydrogen bonds, alkyl bonds, pi-alkyl bonds, carbon hydrogen bonds, covalent bonds, and unfavorable acceptor-acceptor interactions (Fig. 4C). The potential binding sites of oleanolic acid and *CYP19A1* include amino acid residues such as ALA-438, ARG-375, MET-303, MET-364, PHE-430, and ARG-115, and the compound forms bonds with amino acids including salt bridges, attractive charges, conventional hydrogen bonds, carbon hydrogen bonds, pi-donor hydrogen bonds, pi-pi T-shaped bonds, amide-pi stacked bonds, alkyl bonds, pi-alkyl bonds, and covalent bonds (Fig. 4D). The potential binding sites of oleanolic acid and *F2* include amino acid residues such as VAL-27, LYS-24, THR-25, LEU-23, TRP-31, and LEU-28, and the compound forms bonds with amino acids like unfavorable bumps, unfavorable donor-donor interactions, alkyl bonds, and pi-alkyl bonds (Fig. 4E). The potential binding sites of oleanolic acid and *HMGCR* include amino acid residues such as LYS-692, ASN-755, LEU-857, HOH-1268, LEU-853, and LEU-562, and the compound forms bonds with amino acids including water hydrogen bonds, conventional hydrogen bonds, unfavorable donor-donor interactions, unfavorable acceptor-acceptor interactions, alkyl bonds, and pi-alkyl bonds (Fig. 4F).

Based on the binding energy results obtained *via* AutoDockTools1-2, a heatmap was generated. The results show that 6 active components of *Vladimiriae Radix* exhibit good docking performance with 6 core targets of BPH, and the active components can form stable bindings with specific amino acid residues of the targets, confirming that the two have good binding activity Especially for oleanolic acid, its binding energy with various proteins is better than other active compounds, which is also the reason why we will continue to choose it for *vitro* experimental verification in the next step (Fig. 4G).

3.7 Cellular Experiment Results

3.7.1 Effect of Oleanolic Acid on the Proliferation of BPH-1 Cells

Microscopic image analysis showed that compared with the blank control group, BPH-1 cells in the model group proliferated significantly. After treatment with oleanolic acid at different concentrations (10–40 μM), the cell survival rate decreased, with a significant inhibitory effect in the oleanolic acid groups ($p < 0.05$), and the positive drug group was also effective (Fig. 5A). CCK-8 assay results indicated that compared with the blank control group, the proliferative activity of BPH-1 cells in the model group established with 500 nM DHT was significantly increased ($p < 0.05$). After treatment with 10–40 μM oleanolic acid, the cell survival rate was reduced, among which the 10 μM, 20 μM, and 40 μM oleanolic acid groups showed significant inhibitory effects ($p < 0.05$), and the positive drug group was also effective (Fig. 5B).

3.7.2 Effect of Oleanolic Acid on mRNA Expression of BPH-Related Genes in BPH-1 Cells

To clarify the mechanism of action of oleanolic acid on BPH-1 cells, RT-PCR was used to detect the mRNA expression levels of *AR*, *CYP17A1*, *CYP19A1*, *ACHE*, *F2*, and *HMGCR* genes. The results showed that compared with the control group, the mRNA expression levels of *AR* and *HMGCR* in the BPH-1 cell model group established with 500 nM DHT were significantly increased ($p < 0.05$), the mRNA expression levels of *CYP17A1*, *CYP19A1*, and *ACHE* were significantly decreased ($p < 0.05$), and there was no significant change in *F2* expression. Compared with the model group, all oleanolic acid treatment groups (10 μM, 20 μM, 40 μM) significantly downregulated the mRNA expression of *AR* and *HMGCR* ($p < 0.05$), significantly upregulated the mRNA expression of *CYP17A1*, *CYP19A1*, and *ACHE* ($p < 0.05$), and had no significant effect on *F2* (Fig. 5C). These results suggest that oleanolic acid may inhibit the proliferation of BPH-1 cells by regulating the expression of the aforementioned key genes.

4. Discussion

To systematically elaborate the scientific mechanism underlying the anti-BPH effect of the dried roots of *Vladimiriae Radix*, this study employed network pharmacology and molecular docking techniques as core methods. From the three-dimensional perspective of "component-target-pathway", it explored the pharmacodynamic material basis and action logic of D. souliei. This not only addresses the practical challenges in clinical treatment of BPH but also provides a new paradigm for the modernization research on the integrated application of Tibetan medicine and TCM [17]. BPH, a common benign disease of the urinary system in elderly men, exhibits distinct "multidimensional intertwined" characteristics in its pathological mechanism [18]. Specifically, the uncontrolled prolifera-

tion of epithelial and stromal cells in the prostatic transition zone represents the core pathological manifestation [19], the imbalance of the hormonal microenvironment mediated by DHT serves as the key driving factor [20], and chronic non-bacterial inflammatory infiltration and dysregulation of urethral cholinergic nerve modulation respectively exacerbate tissue damage and LUTS [21]. These three factors together form the complex pathological network of BPH. In current first-line clinical treatment regimens, although α-adrenergic blockers can rapidly relax urethral smooth muscle to relieve dysuria, they are prone to causing adverse reactions such as orthostatic hypotension and dizziness [22]. While 5α-reductase inhibitors can reduce prostate volume by decreasing DHT production, they are associated with issues like decreased libido and drug tolerance [23]. The limitations of such "single-target intervention" [24] highlight the urgency of exploring multi-target, low-toxicity treatment strategies. In TCM, BPH is categorized under the scope of "Long Bi" (dysuria and anuria syndrome). Its core pathogenesis is defined as "kidney deficiency as the root cause, dampness-heat as the superficial symptom, and blood stasis as the pathological change" [25]. Notably, the characteristic of TCM involving the synergistic effect of multiple components is highly compatible with the pathological complexity of BPH. *Vladimiriae Radix*, a characteristic medicinal herb used in Tibetan medicine for "promoting qi circulation and dredging collaterals" and in TCM for "strengthening the spleen and harmonizing the stomach", has been the focus of previous studies mostly on the analgesic, anti-inflammatory, and anti-tumor activities of its sesquiterpene lactone components. However, a systematic understanding of its mechanism of action against BPH has not yet been established, which also serves as the core research direction of this study [26]. In the present study, through the combined retrieval of multiple databases including TCMSP, PubChem, and HERB, and combined with the strict evaluation of gastrointestinal absorption rate, DL, Analytical data ensuring that the six finalized compounds (costunolide, dehydrocostus lactone, luteolin, quercetin, oleanolic acid, taraxasterol) possess favorable pharmacokinetic profiles. This provides a clear direction for the material basis of *Vladimiriae Radix* in anti-BPH. Among these components, costunolide and dehydrocostunolide, as the sesquiterpene lactone components with the highest content in *Vladimiriae Radix* [27], have the Δ11(13) exocyclic double bond in their molecular structures, which has been found to be a key functional domain for regulating cell proliferation. Previous studies have shown that these two components can inhibit the proliferative activity of tumor cells such as breast cancer MCF-7 and cervical cancer HeLa by disrupting the stability of microtubule polymerization and inducing the ROS-mediated apoptotic pathway. Considering the commonality in the pathological feature of "uncontrolled cell proliferation" between BPH and tumors [28], it is hypothesized that these components may target and inhibit the abnormal proliferation of prostate cells through similar molecular mechanisms. This hypothesis has also been initially supported by the subsequent molecular docking results. In addition, oleanolic acid, a pentacyclic triterpenoid component, can reduce the release of inflammatory factors such as IL-6 and tumor necrosis factor-alpha (TNF-α) by inhibiting the nuclear factor-kappa B (NF-κB) inflammatory signaling pathway, thereby specifically improving the chronic inflammatory microenvironment of BPH [29]. Quercetin, a flavonoid component, can correct the imbalance of estrogen and androgen ratios in prostate tissue by regulating the activity of estrogen receptors [30]. Meanwhile, phytosterols such as stigmasterol and β-sitosterol can indirectly regulate the local hormone levels in the prostate by interfering with cholesterol metabolism (the precursor pathway for steroid hormone synthesis) [31]. These 6 components cover the three core pathological links of BPH, namely "proliferation inhibition, inflammation alleviation, and hormone regulation". This not only reflects the diversity of the pharmacodynamic substances of *Vladimiriae Radix* but also echoes the concept of the "monarch-minister-adjuvant-courier" synergistic effect in TCM. The excavation and validation of core targets further suggest the pivotal role of *Vladimiriae Radix* against BPH. Through database cross-analysis and the construction of a PPI network, this study screened 235 overlapping targets from 342 active targets of *Vladimiriae Radix* and 5256 disease targets of BPH. Based on degree value and topological analysis, 6 core targets (*AR*, *CYP17A1*, *CYP19A1*, *ACHE*, *F2*, and *HMGCR*) were identified, and these targets exactly correspond to the key nodes in the pathological mechanism of BPH. Among these, the *AR* is the "hub target" for BPH pathogenesis [32]. Specifically, the specific binding of DHT to *AR* can activate the expression of downstream proliferation-related genes such as MYC and CCND1, thereby promoting the continuous proliferation of prostate cells. Clinically, the drug finasteride achieves "indirect regulation" of *AR* by inhibiting 5α-reductase to reduce DHT production [33]. However, the molecular docking results of this study showed that costunolide and dehydrocostunolide can form hydrogen bonds, hydrophobic interactions, and covalent bonds with active sites such as LEU-93, ILE-119, and TRP-189 of *AR*. This suggests that these components may directly block the formation and nuclear translocation of the DHT-AR complex by competitively binding to the ligand-binding domain of *AR*. Compared with clinical drugs, this "direct intervention" mode may have stronger target specificity. Cytochrome P450 enzyme family members *CYP17A1* and *CYP19A1* jointly regulate steroid hormone metabolism [34], *CYP17A1*, as the rate-limiting enzyme in androgen synthesis, an abnormal increase in its activity leads to local androgen accumulation in the prostate [35], *CYP19A1* (aromatase) can convert testosterone to estradiol, and the imbalance of estrogen and androgen ratios induced by its increased activ-

ity further promotes AR expression and stromal cell proliferation [36]. This study found that *Vladimiriae Radix* components can bind to the active sites of *CYP17A1* (such as PHE-446 and CYS-453) and *CYP19A1* (such as ALA-306 and ARG-375). It is hypothesized that these components may correct hormone imbalance at the source by exerting dual inhibition on the activity of these two enzymes. Compared with abiraterone, which only inhibits *CYP17A1*, this "dual-target regulation" mode may reduce the risk of adverse reactions while regulating hormone balance. The identification of *ACHE*, *F2*, and *HMGCR* further expands the mechanistic dimensions of *Vladimiriae Radix* in anti-BPH. *ACHE* maintains cholinergic nerve signal balance by degrading acetylcholine in the synaptic cleft [37]. Abnormal elevation of its activity leads to excessive contraction of urethral smooth muscle, which exacerbates bladder outlet obstruction and LUTS. This study shows that *Vladimiriae Radix* components can bind to the catalytic active sites of *ACHE* (such as CYS-345 and SER-347). It is hypothesized that these components may increase acetylcholine concentration by inhibiting *ACHE* activity, thereby enhancing the regulation of urethral smooth muscle relaxation. This mechanism is complementary to that of solifenacin, a clinical anticholinergic drug, and the nature of natural components may reduce the risk of central nervous system adverse reactions. Factor II (*F2*), a key factor in the coagulation-inflammation pathway, can promote the release of inflammatory factors by activating protease-activated receptors (PARS), thereby exacerbating inflammation and fibrosis in prostate tissue [38]. As the rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*HMGCR*) not only affects steroid hormone synthesis, but its abnormal activity is also closely associated with oxidative stress and cell proliferation in prostate tissue [39]—clinical studies have found that statins can reduce the risk of BPH development by inhibiting *HMGCR*. The good binding activity of *Vladimiriae Radix* components to *F2* and *HMGCR* suggests that these components may intervene in the progression of BPH from two dimensions—the inflammatory microenvironment and metabolic abnormalities—by regulating the coagulation-inflammation pathway and lipid metabolism pathway. This further improves the "multi-target synergy" mechanism of *Vladimiriae Radix*. GO functional annotation and KEGG pathway enrichment analyses preliminarily corroborated, at the systems level, the multi-dimensional characteristics of *Vladimiriae Radix* against BPH. At the BP level, core targets are enriched in terms such as steroid hormone response and vascular processes, which are highly consistent with the hormone-dependent characteristics of BPH and the mechanism of tissue angiogenesis. At the CC level, targets are concentrated in structures including membrane rafts and membrane microdomains. As aggregation platforms for signaling molecules (*e.g.*, AR, G protein-coupled receptors), dysfunction of membrane rafts is a key link in the loss of control of BPH pathological signals—suggesting that *Vladimiriae Radix* components may inhibit signal transmission by interfering with membrane raft structure [40]. At the MF level, G-protein-coupled amine receptor activity and acetylcholine receptor activity were significantly enriched, further suggesting that *Vladimiriae Radix* intervenes in BPH by modulating receptor activity. The results of KEGG pathway enrichment analysis showed that the core targets are mainly involved in pathways such as lipid and atherosclerosis, cholinergic synapses, and AGE-RAGE. Specifically, lipid metabolism disorders can exacerbate BPH through cholesterol deposition, the cholinergic synapses pathway directly corresponds to the mechanism of action of *ACHE* [41], and the AGE-RAGE pathway is closely associated with BPH tissue fibrosis [42]. The synergistic enrichment of these pathways fully reflects the characteristic of *Vladimiriae Radix* in intervening in BPH through a "multi-pathway and multi-link" manner, and also provides a modern biological explanation for the advantage of "treating both the root cause and symptoms" (a key principle of TCM). Molecular docking results further support the inference that the active components of *Vladimiriae Radix* possess specific binding capabilities to the core targets. The binding sites of components such as costunolide and dehydrocostunolide with targets including AR, *CYP17A1*, and *ACHE* are all located in the active center regions of these targets, and binding is achieved through stable interactions such as hydrogen bonds, hydrophobic interactions, and salt bridges. For instance, there is a hydrogen bond formed between costunolide and TRP-189 of *AR*, and a salt bridge formed between costunolide and ARG-109 of *CYP17A1*. Both the type and strength of these interactions meet the key indicators of molecular binding activity, providing a basis for *Vladimiriae Radix* components to serve as candidate compounds for BPH treatment. Differences in the binding modes between different components and the same target (*e.g.*, the number of hydrogen bonds formed between quercetin and *ACHE* is greater than that between costunolide and *ACHE*) suggest that there may be potential for synergistically enhancing target regulation among these components. This is also consistent with the characteristic of the synergistic effect of multiple components in TCM. The innovative value of this study is mainly reflected in two aspects: Firstly, it constructs the "component-target-pathway" systematic network of *Vladimiriae Radix* for anti-BPH from the perspective of network pharmacology for the first time, clarifies its core active components and key functional nodes, and fills the research gap of *Vladimiriae Radix* in the field of BPH treatment [43]. Secondly, it realizes the cross-integration of Tibetan medicine characteristic medicinal materials and modern pharmacology technologies, and provides reference methodological ideas for the mechanism research of ethnic medicine in the treatment of urinary system diseases. However, this study still has limitations. Firstly, network pharmacology relies on the integrity of

existing databases, which may miss components with low content but high activity or unannotated targets. Secondly, molecular docking is only an *in vitro* computer simulation, lacking functional verification from *in vivo* BPH animal models and *in vitro* cell experiments, and thus cannot clarify the actual regulatory effect of core components on targets. Furthermore, the impact of *Vladimiriae Radix* processing techniques on active components has not been incorporated into the study. Processing is a crucial link for TCM to exert its efficacy and may alter its pharmacodynamic material basis. In summary, the present study preliminarily proposes that *Vladimiriae Radix* exerts anti-BPH effects through the synergistic action of "multi-component, multi-target, and multi-pathway" mechanisms, identifying its core active ingredients and key therapeutic targets. These findings provide experimental support for both basic research and clinical application of *Vladimiriae Radix*, and offer a new perspective for investigating the mechanisms of traditional Chinese medicine in BPH treatment. Further experimental validation is needed to refine this mechanism and promote the translational application of *Vladimiriae Radix* in BPH therapy.

5. Conclusion

Costunolide, dehydrocostus lactone, oleanolic acid, luteolin, quercetin, and taraxasterol are bioactive components of *Vladimiriae Radix* with potential anti-BPH properties. *AR*, *CYP17A1*, *CYP19A1*, *ACHE*, *F2*, and *HMGCR* were the core targets which related to the bioactive components in anti-BPH. Oleanolic acid exhibits the good binding energy with 6 core targets. Cell-level verification showed that oleanolic acid may significantly inhibits the proliferation of DHT-induced BPH-1 cells ($p < 0.05$) and regulates the mRNA expression of core targets (downregulating *AR* and *HMGCR*, upregulating *CYP17A1*, *CYP19A1*, and *ACHE*, with no significant effect on *F2*). In summary, the study found that *Vladimiriae Radix* exerts anti-BPH effects through a "multi-component, multi-target, and multi-pathway" mode of action, filling the gap in the anti-BPH mechanism research of *Vladimiriae Radix* and providing reliable experimental evidence for the deepening of its basic research, promotion of clinical application, and reference for the modernization of Tibetan-Chinese integrated medicinal materials.

Availability of Data and Materials

The datasets generated and analysed during the current study are available upon request from the corresponding author.

Author Contributions

JXM conceived and designed the research. XDF, JW, and JXM carried out the data analysis and wrote the paper in the present study. XDF and JW finished the drawing and experiment. JXM carried out the manuscript revision work. All authors participated in the editing and revision of the manuscript. All authors have read and approved the final manuscript. All authors fully participated in this research and agree to be accountable for all aspects of the study.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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