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RESEARCH

Network pharmacology and experimental validation to explore the pharmacological mechanism of saw palmetto and its core ingredients in benign prostatic hyperplasia treatment

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Abstract

Benign prostatic hyperplasia (BPH) is a prevalent urological condition that predominantly affects the geriatric male population, resulting in lower urinary tract symptoms. Saw palmetto is a traditional Chinese medicine for treating BPH. This study aimed to investigate the potential therapeutic mechanisms of saw palmetto in BPH treatment. The active ingredients and potential targets of saw palmetto were obtained through the TCMSP database. BPH-related targets were retrieved from the GeneCards database. PPI, GO, and KEEG analyses were performed to predict the potential therapeutic mechanism. The active ingredient-common target and common target-pathway networks were constructed by Cytoscape software. Molecular docking and cellular experiments were carried out to further validate the potential mechanism. We obtained 13 active components in saw palmetto and 56 common targets in BPH treatment. KEEG analysis showed that the estrogen signaling pathway was the most enriched and exhibited a close association with BPH. PPI analysis, along with ingredient-target and target-pathway network analyses, indicated that stigmasterol was the core ingredient and *PGR*, *NCOA1*, and *NCOA2* were identified as the hub genes mediating the effects of saw palmetto against BPH. In addition, molecular docking showed that stigmasterol had strong binding to *PGR*, *NCOA1*, and *NCOA2*. Cellular experiments revealed that stigmasterol significantly increased the percentage of BPH-1 cells in the G0/G1 phase and inhibited cell viability and division. Furthermore, it notably reduced the expression of *PGR*, *NCOA1*, and *NCOA2*. Saw palmetto might inhibit cell viability and division by suppressing the expression of *PGR*, *NCOA1*, and *NCOA2*, thereby playing a therapeutic role in treating BPH.

Keywords Benign prostatic hyperplasia · Saw palmetto · Network pharmacology · Molecular docking · Cellular experiments

Introduction

Benign prostatic hyperplasia (BPH), a prevalent urological condition predominantly affecting the geriatric male population, is characterized by the non-malignant proliferation of prostatic cells. This condition primarily impacts the periurethral region, resulting in clinical manifestations such as urethral obstruction and painful urination (Miernik and Gratzke 2020). Although the pathogenesis of BPH remains unclear, it is known to be related to age, hormonal fluctuations, and genetic factors (Devlin et al. 2021). BPH significantly affects the quality of life of patients, manifesting in symptoms such as frequent micturition, urgent micturition, and excessive urination at night. Even worse, it may lead to urinary retention and renal function damage (Launer et al. 2021). Currently, the treatment strategies for BPH mainly include drug treatment, minimally invasive

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surgery, and traditional surgery (Miernik and Gratzke 2020). Drug treatment mainly aims to relieve symptoms and shrink the prostate through α receptor antagonists and 5α-reductase inhibitors. However, this approach has certain limitations, such as slow onset of effect, long-term use, and possible side effects (Joseph et al. 2022). Alternatively, surgery can effectively alleviate symptoms but carries the risk of complications like postoperative sexual dysfunction and urinary incontinence (Khooblall et al. 2023). With the aging of the global population, the prevalence rate of BPH is expected to continue to rise, posing a huge pressure on the public health system (Launer et al. 2021). Therefore, it is of great practical significance to explore novel treatment strategies and drugs for BPH, which will not only improve the quality of life of elderly men but also reduce the burden on the medical system.

In the theory of traditional Chinese medicine (TCM), BPH is usually regarded as a symptom of "dysuria" or "spermatorrhea," associated with deficiencies in kidney qi, stagnation of qi and blood, and internal accumulation of damp heat (Wen et al. 2024). The treatment of BPH in TCM primarily focuses on harmonizing qi and blood, replenishing kidney energy, and clearing heat and dampness, to alleviate symptoms and improve the quality of life (Huang et al. 2020). TCM pays attention not only to the relief of symptoms but also to the eradication of etiology and the restoration of the body's overall balance of the body (Li et al. 2023a). In addition, Chinese medicine can make personalized treatment plans to address individual differences. At present, TCM has shown certain potential in the treatment of BPH and can achieve significant therapeutic effects with high efficacy and low toxicity (Zhao et al. 2021; Jin et al. 2019; Tran et al. 2022). Hence, the exploration and application of TCM can provide invaluable clues for developing new therapeutic drugs for BPH.

Saw palmetto, also known as *Serenoa repens*, is a common herbal plant native to the southeastern region of North America. The fruit of the saw palmetto is utilized in the production of herbal supplements and is also widely employed in TCM for the treatment of specific conditions such as BPH and urinary tract issues (Marks and Tyler 1999). Saw palmetto contains numerous components, such as fatty acids, phytosterols, and polyphenols, which play a pharmacological role in anti-inflammation, antiandrogenic activity, and enhancement of urodynamics (Gong and Gerber 2004). Saw palmetto has been widely used in the treatment of BPH, which can inhibit the enzyme 5α-reductase, leading to a reduction in the production of dihydrotestosterone, thereby slowing down the proliferation of prostate cells (Kwon 2019). Several studies have demonstrated the potential of saw palmetto in treating BPH. For instance, Marks and Tyler had discussed saw palmetto extract as a new (and oldest) alternative therapy for symptomatic BPH in their 1999 review (Marks and Tyler 1999). Kwon indicated that saw palmetto extract had a positive effect on BPH (Kwon 2019). Sudeep et al. revealed that saw palmetto oil, abundant in phytosterols, had similar efficacy in alleviating BPH and androgen deficiency when compared to traditional saw palmetto oil (Sudeep et al. 2020). Moreover, *Serenoa repens* has demonstrated good tolerability and has shown efficacy similar to α-blockers in improving voiding and storage symptoms, increasing urinary flow rate, and reducing prostate volume in men with BPH (Blair 2022). Nevertheless, the specific therapeutic mechanisms of saw palmetto in BPH treatment remain uncertain.

Network pharmacology emerges as a new interdisciplinary realm, integrating the theories and methods of systems biology, pharmacology, informatics, and network science. It is devoted to studying the intricate interaction between drugs and diseases (Nogales et al. 2022). Unlike traditional single-drug target research, network pharmacology offers a holistic analysis of network-based relationships among drug components, targets, and diseases, unveiling the overall and systematic characteristics of drug action. This comprehensive approach provides a fresh perspective and tool for studying the molecular mechanisms of TCM against diseases (Jiashuo et al. 2022). Network pharmacology helps us to deeply understand the complex mechanism of action of TCM by constructing a network model that interconnects drug components, targets, and diseases. Furthermore, the results obtained from this approach can serve as a robust foundation for subsequent experimental endeavors (Zhou et al. 2020).

In this study, we applied network pharmacology to screen the core active ingredients, core targets, and relevant signaling pathways of saw palmetto in BPH treatment. Subsequently, the interactions between these core ingredients and related targets were verified through molecular docking analysis. The potential therapeutic mechanism of saw palmetto against BPH was further validated by cellular experiments. The flowchart of this study is depicted in Fig. 1.

Materials and methods

Screening the active ingredients and targets of saw palmetto

The active components of saw palmetto were obtained from the publications. Subsequently, all active components were input into the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (https://old.tcmsp-e.com/index.php) to predict the targets associated with the potential active ingredients of saw palmetto. These targets were then converted into corresponding gene names using the UniProt database (https://www.uniprot.org/).

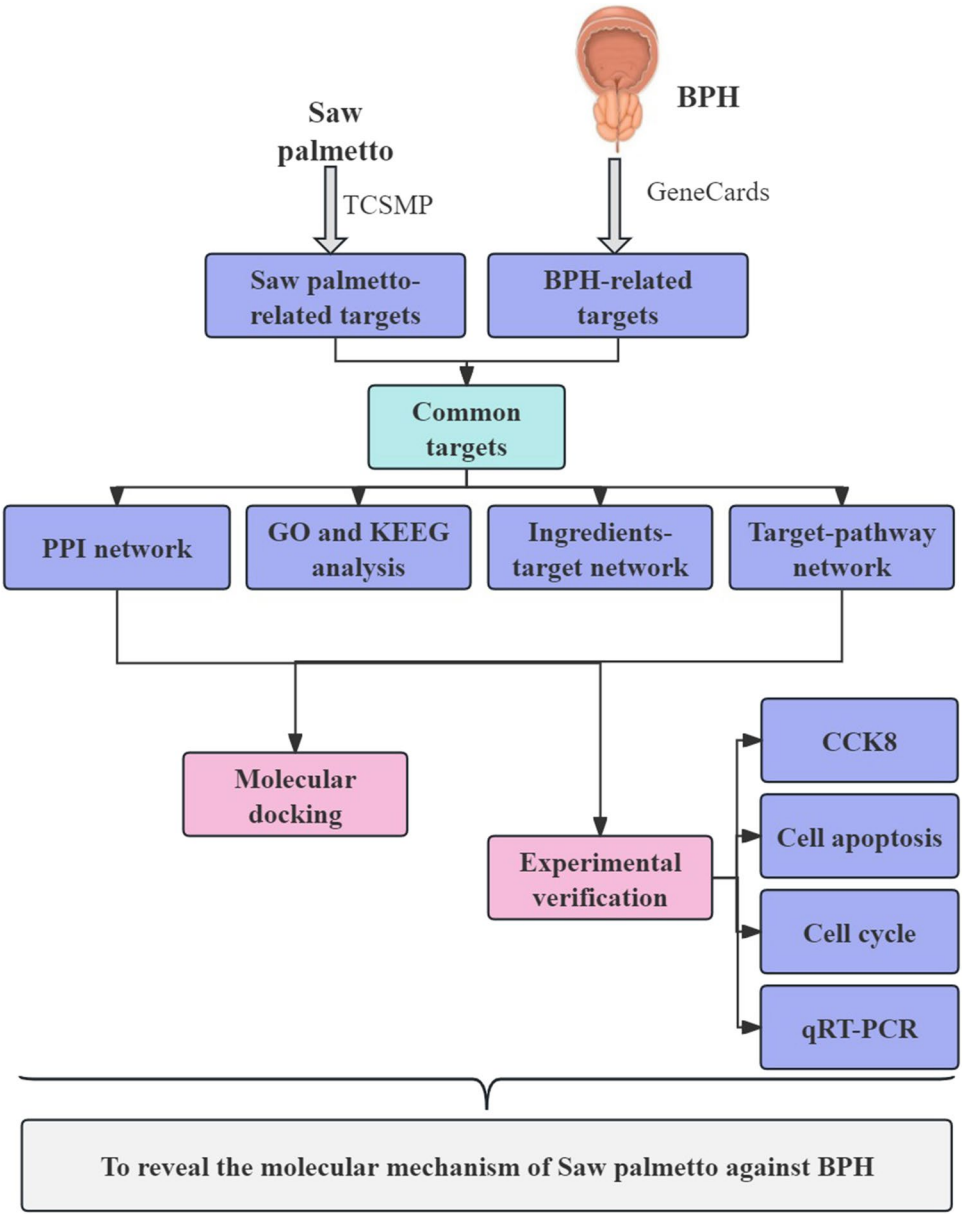

Fig. 1 Flow diagram of the study of saw palmetto in BPH treatment. BPH, benign prostatic hyperplasia; GO, Gene Ontology; KEEG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein–protein interaction; TCSMP, Traditional Chinese Medicine Systems Pharmacology database and analysis

BPH target prediction

We screened the targets related to BPH through the GeneCards database (https://www.genecards.org/) with "Benign prostatic hyperplasia" as the keyword, and these targets were then standardized through the UniProt database to obtain their gene names and identifiers.

Screening the potential therapeutic targets of saw palmetto in BPH treatment and constructing a PPI network

To obtain the potential therapeutic targets of saw palmetto against BPH, we imported saw palmetto and disease targets into the Venny 2.1 online tool (http://liuxiaoyuyuan.cn/). The overlapping targets were the potential therapeutic targets of saw palmetto in BPH treatment. These common targets were then imported into the STRING database (http://string-db.org/) for protein–protein interaction (PPI) analysis. Cytoscape 3.10.0 was performed to determine the key therapeutic targets according to the degree value.

GO and KEEG analyses

To explore the therapeutic mechanism of saw palmetto in BPH treatment, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of these common targets were conducted through the Hiplot database (http://hiplot.com.cn/). The GO enrichment analysis revealed genes associated with biological processes (BP),

cellular compositions (CC), and molecular functions (MF), whereas the KEGG enrichment analysis primarily focused on identifying pathway-associated information.

Construction of herb-active ingredient-common target-disease and target-signaling pathway networks

The Cytoscape 3.9.1 software was employed to construct the networks that demonstrate the associations between the herb, active ingredients, targets, and pathways. In these networks, nodes represent active ingredients, targets, or pathways, while edges represent their interactions. The degree value of a node indicates the number of edges connected with the node, and the core component or path of the node is determined by the highest degree value.

Molecular docking

Molecular docking, based on structural analysis, is a crucial technique for drug design and screening. It enables the prediction of binding affinity and mode between ligand and receptor molecules, making it a common method in drug research (Pinzi and Rastelli 2019). In the present study, we performed molecular docking to preliminarily validate the core active ingredients and therapeutic targets identified through network pharmacology. The three-dimensional protein structures were retrieved from the RCSB Protein Data Bank database (PDB, http://www.rcsb.org/), while the molecular structures of the active ingredients were sourced and downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The molecular docking analysis was conducted by Molecular Operating Environment (MOE) software. In this analysis, target-ligand pairs with a selection value of ≤ -5 kcal/mol were deemed to possess moderate to strong binding affinity (Wang et al. 2022; Guo et al. 2023). Notably a lower binding energy indicates a higher binding efficacy between the molecules.

In vitro experimental validation

Preparation of stigmasterol solution

Stigmasterol (HPLC $\geq$ 98%), purchased from MedChemExpress, was dissolved in DMSO to achieve a final concentration of 10 mM for further applications.

Cell culture

Human BPH-derived prostate epithelial cell lines (BPH-1) were purchased from Otwo Biotech (Otwo, Shenzhen, China). The cells were cultured in DMEM (PromoCell, Wuhan, China) medium supplemented with high-quality fetal bovine serum (Sigma, F8313) and 1% penicillin/streptomycin (PromoCell, Wuhan, China) at 37 °C with 5% CO_2 and 95% air.

Cell viability assay

The viability of BPH-1 cells was assessed by Cell Counting Kit-8 (CCK8) (Beyotime, Hangzhou, China) following the manufacturer's protocol. Initially, the cells were plated in a 96-well microplate at a concentration of 1×10^4 cells per well and cultured in an incubator at 37 °C with 5% CO_2 for 24 h. Once the cells had fully attached, they were treated with stigmasterol at concentrations of 0, 10, 25, 100, 200, and 500 μM for 24 h. After pre-incubation in a cell culture incubator, the medium was aspirated, and each well was replenished with 100 μL of the prepared CCK8 working solution. The plate was then returned to the incubator at 37 °C for further incubation until the color change to orange was observed. Subsequently, the absorbance was quantified at 450 nm using a microplate reader. The cell proliferation rate was determined using the following formula: $(OD_{Experimental\ Group} - OD_{Control\ Group})/(OD_{Control\ Group} - OD_{Control\ Group}) \times 100\%$.

Flow cytometry analysis of apoptosis

BPH-1 cells were inoculated into a 6-well plate at a density of 1×10^5 cells/well and cultured for 24 h in a 37 °C environment with 5% CO_2. After washing with PBS, the cells were collected and centrifuged at 2000 rpm for 5 min. The supernatant was discarded, and the cells were washed again with PBS, followed by another centrifugation at the same speed and duration. The supernatant was then discarded, and 100 μL of a 1% BSA solution was added. Subsequently, 100 μL of cell suspension, adjusted to a concentration of 2×10^5 to 1×10^6 cells/mL, was combined with 100 μL of Muse Annexin V & Dead Cell reagent (Luminex, USA) in a 1.5-mL centrifuge tube. After incubation in the dark at room temperature for 20 min, the stained samples were filtered and analyzed using flow cytometry (Muse, USA).

Cell cycle assay

Cells were plated and treated as described above. At 24 h post-treatment, they were harvested, rinsed with PBS, and trypsinized with 0.25% trypsin for 2 min. Fetal bovine serum was added to neutralize the trypsin, and the cells were collected by centrifugation at 1000 rpm for 5 min. After discarding the supernatant and washing with PBS, the cells were fixed in 70% ethanol and stored at − 4 °C. For analysis, cells were centrifuged again at 2000 rpm for 5 min, the ethanol was removed, and the cells were washed with PBS. They were then stained with DAPI in the dark

for 20 min, followed by a final wash and resuspension in PBS. Samples were analyzed through flow cytometry.

qRT-PCR analysis

Total RNA was isolated from cell samples using the TRIzol reagent (AgBio, Hunan, China), following the manufacturer's instructions. RNA concentration and integrity were assessed using a NanoDrop 2000 spectrophotometer. Subsequently, the RNA of sufficient quality was subjected to reverse transcription with the NovoScript® Plus 1st Strand cDNA Synthesis SuperMix. qRT-PCR was performed using the SYBR High-Sensitivity qPCR SuperMix (Novoprotein, Suzhou, China) on an ABI 7500 Real-Time PCR system. GADPH served as the endogenous control for normalization. The primer sequences utilized in the qRT-PCR reactions are detailed in Table 1.

Table 1 Primer sequences used for quantitative real-time PCR

Gene name	Primer	Sequence (5′ to 3′)
PGR	Forward primer	GTCGCCTTAGAAAGTGCTGTCAG
	Reverse primer	GCTTGGCTTTCATTTGGAACGCC
NCOA1	Forward primer	ATCGGAGATCCTGCCAGCTTCA
	Reverse primer	ATCGCCTGTTCCTGGTTGTCCA
NCOA2	Forward primer	GCCCAGAAAACAGCACTGCGAA
	Reverse primer	CTGATTGGTGGGAAAGGTCCAG
GAPDH	Forward primer	GGAGCGAGATCCCTCCAAAAT
	Reverse primer	GGCTGTTGTCATACTTCTCATGG

Statistical analysis

In this research, data analysis was conducted using GraphPad Prism version 9.0. Results were presented as mean ± standard deviation (SD). Statistical comparisons across and within groups were performed using one-way analysis of variance (ANOVA). A p-value of less than 0.05 was considered statistical significance.

Results

Potential active ingredients and targets of saw palmetto

In this study, we screened 13 active components of saw palmetto by consulting relevant literature (Table 2). These included stigmasterol, linolenic acid, lauric acid, oleic acid, linoleic, palmitic acid, beta-sitosterol, myristic acid, stearic acid, campesterol, caprylic acid, palmitoleic acid, and capric acid. After excluding overlapping targets, we ultimately obtained 72 targets for these active ingredients through the TCSMP database.

Therapeutic target prediction for saw palmetto in the treatment of BPH

We obtained 4280 potential BPH-related targets by searching the GeneCards database. A Venn diagram of disease-related targets and saw palmetto targets was subsequently constructed, and 56 intersecting targets were selected as potential therapeutic targets for saw palmetto in BPH treatment (Fig. 2A). To obtain the key therapeutic targets, the

Table 2 Saw palmetto ingredients and their corresponding targets

Molecular ID	Molecular name	Targets number	Targets
MOL000449	Stigmasterol	20	PGR/NR3C2/NCOA2/RXPA/NCOA1/PTGS1/PTGS2/ADRA2A/ADRB2/AKR1B1/PLAU/LTA4H/MAOB/MAOA/CTRB1/CHRM3/ADRB1/ADRA1A/CHRM2/ADRA1B
MOL000432	Linolenic acid	13	PTGS1/PTGS2/RXRA/NCOA2/RXRG/TRPV1/RELA/PCNA/ALB/MYC/HNF4A/UCP2/HNF1A
MOL000305	Lauric acid	12	PTGS1/BCHE/PLA2G4F/PTGS2/RELA/AKT1/IL6/CXCL8/DUOX2/CD80/CD86/CD40
MOL001308	Oleic acid	12	PTGS1/PTGS2/RXRA/NCOA2/RHO/CHRM3/KCNH2/ADRA1B/ADRB2/ADRA1D/RXRB/PGR
MOL000749	Linoleic	11	PTGS1/PTGS2/RXRA/NCOA2/ADRB1/ADRA2A/CHRM2/ADRA2B/ADRB2/SLC6A4/MAOB
MOL000069	Palmitic acid	9	CTSD/PTGS1/PTGS2/RHO/NCOA2/BCL2/IL10/TNFSF15/COL1A1
MOL000358	Beta-sitosterol	7	BAX/BCL2/CASP3/CASP8/JUN/PGR/PTGS2
MOL001393	Myristic acid	6	PTGS1/PTGS2/BCHE/PLA2G4F/NCOA2/NCOA1
MOL000860	Stearic acid	5	PTGS1/PTGS2/RXRA/NCOA2/SP1
MOL000493	Campesterol	4	PGR/PTGS1/PTGS2/NCOA2
MOL000303	Caprylic acid	4	PLA2G4F/CXCL8/PPARA/EP300
MOL001739	Palmitoleic acid	3	PTGS1/PTGS2/NCOA2
MOL001640	Capric acid	1	PLA2G4F

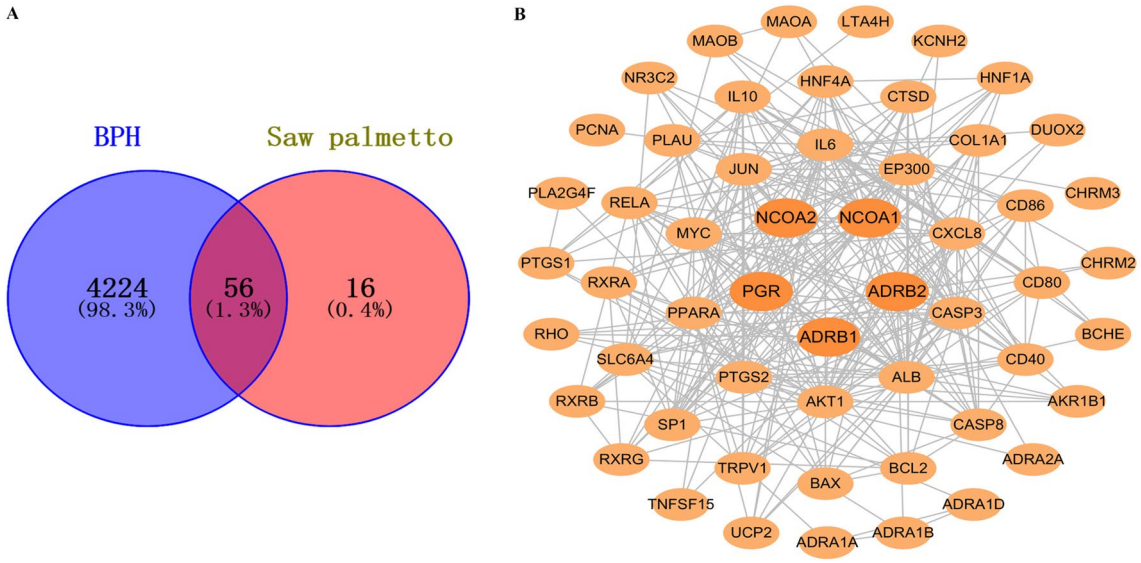

Fig. 2 Screen for the potential therapeutic targets in saw palmetto against BPH. **A** Venn diagram of common targets of saw palmetto and BPH. **B** PPI network of saw palmetto-BPH target genes

PPI network was constructed based on the common targets, which consisted of 56 nodes and 596 edges. As shown in Fig. 2B, *PGR*, *NCOA1*, *NCOA2*, *ADRB1*, and *ADRB2* might be regarded as the key therapeutic targets with the highest degree value in the PPI network.

GO and KEEG enrichment analyses

GO and KEEG analyses were conducted to determine the therapeutic mechanism of saw palmetto against BPH based on the common targets. The top 20 GO terms and KEEG pathways are presented in the bubble diagram (Fig. 3A–D). Figure 3A illustrates that targets associated with BP are mainly involved in response to steroid hormone, regulation of tube diameter, response to xenobiotic stimulus, response to oxidative stress, and response to extracellular stimulus. In terms of MF (Fig. 3B), these targets are mainly associated with steroid hormone receptor activity, nuclear receptor activity, DNA-binding transcription factor binding, RNA polymerase II–specific DNA-binding transcription factor binding, and ligand-activated transcription factor activity. At the CC level (Fig. 3C), they are mainly related to the transcription regulator complex, organelle outer membrane, outer membrane, membrane raft, and membrane microdomain.

The KEGG pathway analysis highlighted the top 20 most significantly enriched signaling pathways as depicted in Fig. 3D. Among these, the estrogen signaling pathway, which has a significant association with BPH, was chosen for further exploration.

Construction and analysis of ingredient-target and target-signaling pathway networks

To determine the key active ingredient of saw palmetto against BPH, we constructed a network based on disease, herb, active ingredients, and common targets, which consisted of a total of 71 nodes (Fig. 4A). As shown in Table 2, stigmasterol, with the highest degree value (degree = 20) among the active ingredients of saw palmetto, plays a crucial role in treating BPH. In addition, we further constructed a network including stigmasterol and its corresponding targets. As depicted in Fig. 4B, *PGR*, *NCOA1*, *NCOA2*, *ADRB1*, and *ADRB2* exhibited a higher degree value than the other targets. Figure 4C demonstrates that the estrogen signaling pathway contains seven targets, namely *PGR*, *NCOA1*, *NCOA2*, *SP1*, *BCL2*, *JUN*, and *CTSD*.

By integrating the PPI analysis that revealed high degree values for *PGR*, *NCOA1*, *NCOA2*, *ADRB1*, and *ADRB2*, the ingredient-target network that identified stigmasterol as the core ingredient, the KEGG results highlighting the estrogen signaling pathway as the main enrichment pathway related to BPH, and the target-pathway network demonstrating the involvement of *PGR*, *NCOA1*, and *NCOA2* in the estrogen signaling pathway, it can be inferred that saw palmetto, particularly its ingredient stigmasterol, may influence the estrogen signaling pathway by modulating the expression of *PGR*, *NCOA1*, and *NCOA2*, thereby potentially playing a role in BPH treatment.

Molecular docking

To validate the authenticity of molecular interactions with their targets and determine precise binding mechanisms, we

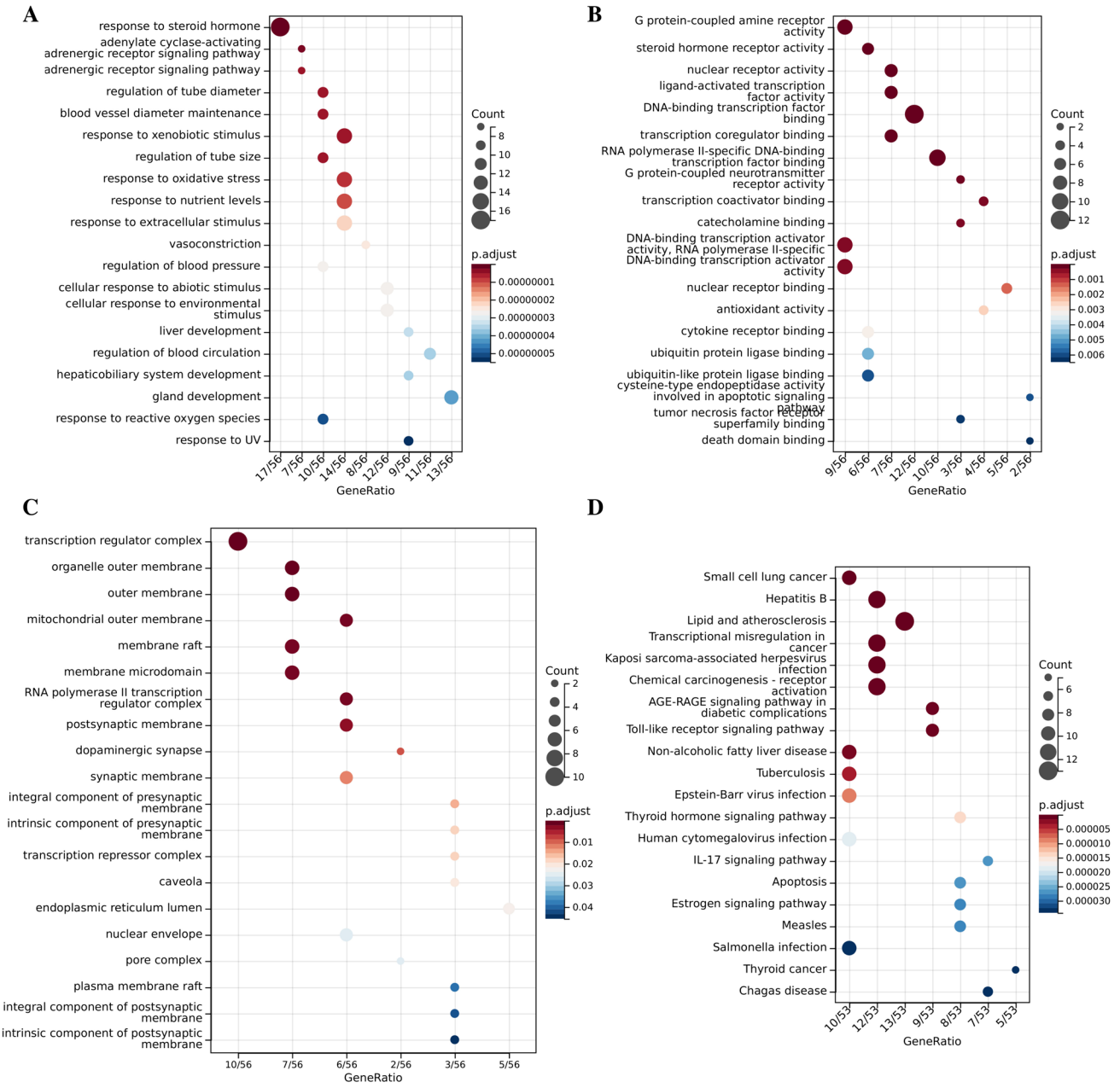

Fig. 3 The diagram for GO and KEEG analyses. **A** Biological process categories. **B** Cellular component categories. **C** Molecular function categories. **D** KEEG enrichment associated with the targets

recognized stigmasterol as the key compound, focusing on *PGR*, *NCOA1*, and *NCOA2* based on the above results. The docking results, presented in Table 3, indicated strong binding affinities for stigmasterol interaction with *PGR* (binding energy = −6.1 kcal/mol), *NCOA1* (binding energy = −6.3 kcal/mol), and *NCOA2* (binding energy = −7.6 kcal/mol), defined by a threshold of −5 kcal/mol or lower.

In Fig. 5A–C, the visualization presents the optimal docking images, where the receptors and ligands with high binding energy have been optimally aligned.

Stigmasterol could inhibit the viability of BPH-1 cells

The CCK-8 assay demonstrated that BPH-1 cell viability was reduced with stigmasterol treatments at concentrations of 100, 200, and 500 μM for 24 h when compared to the control group (all $p < 0.001$), as depicted in Fig. 6A. Among these, the treatments with 100 and 200 μM concentrations for 24 h exhibited the most promising results and were therefore selected for subsequent experiments.

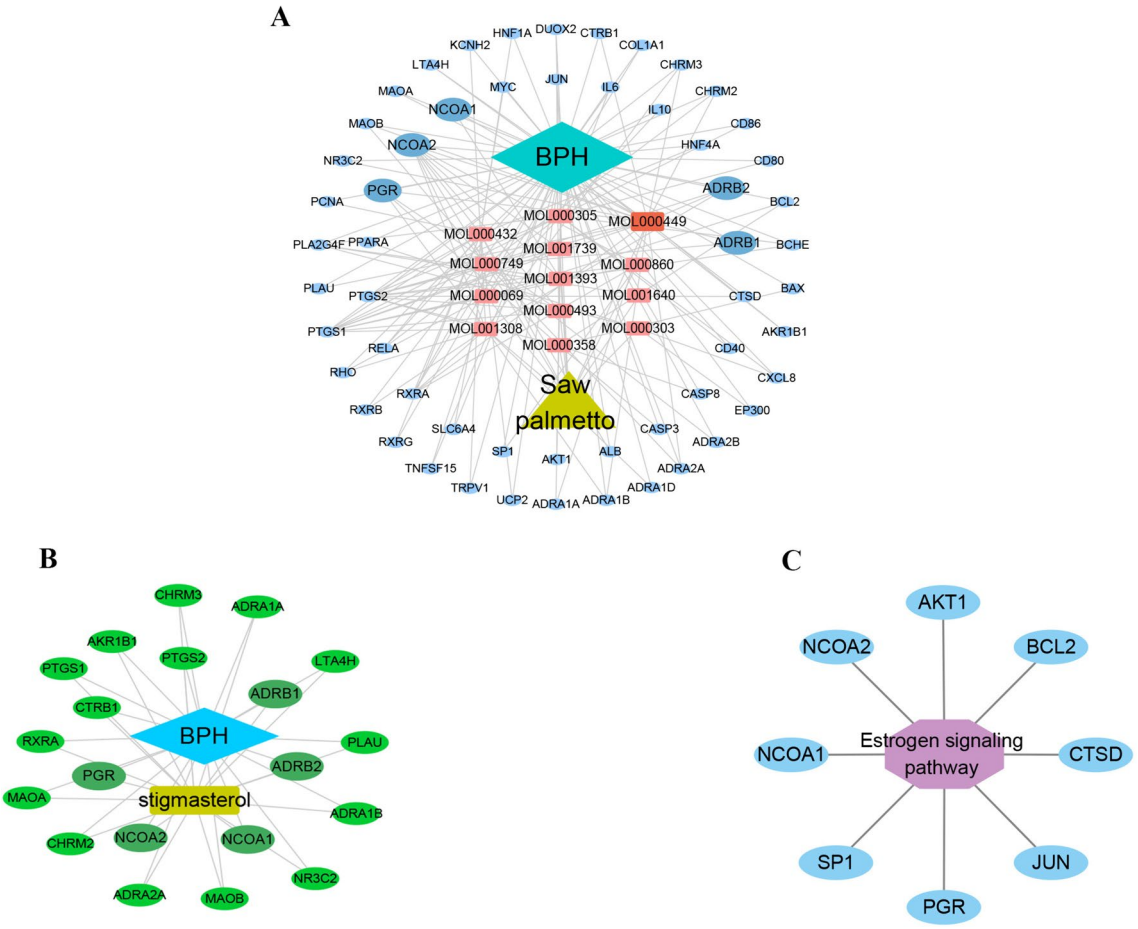

Fig. 4 Network construction of the interaction between herb, active ingredients, targets, and signaling pathway. **A** Herb-active ingredients-targets-disease network. **B** Targets in the critical signaling pathway

Table 3 Molecular docking results of stigmasterol and core targets

Protein	PDB ID	Molecular name	Molecular ID	Affinity (kcal/mol)
PGR	3G8O	Stigmasterol	MOL000449	−6.1
NCOA1	4J5W	Stigmasterol	MOL000449	−6.3
NCOA2	1ZDU	Stigmasterol	MOL000449	−7.6

Effect on cell apoptosis

The flow cytometry experiment was conducted to determine the effect of stigmasterol on cell apoptosis. As shown in Fig. 6B, treatment with stigmasterol could significantly accelerate the cell apoptosis rates of the BPH-1 cells ($p < 0.001$).

Effect on cell cycle

Figure 6C demonstrates that following a 24-h treatment with 100 μM and 200 μM stigmasterol, the experimental group exhibited a higher number of cells in the G0/G1 phase compared to the control group (all $p < 0.001$). Conversely, the percentage of cells in the G2/M phases was notably lower in the stigmasterol-treated group ($p < 0.01$ and $p < 0.001$, respectively). These findings suggest that the drug's application effectively inhibited cell division, thereby controlling BPH-1 cell growth.

Effect of stigmasterol on the mRNA expressions of *PGR*, *NCOA1*, and *NCOA2* in BPH-1 cells

The mRNA expressions of *PGR*, *NCOA1*, and *NCOA2* were evaluated by qRT-PCR, with results shown in Fig. 7A–C. It was observed that the expression levels of *PGR* ($p < 0.05$), *NCOA1* ($p < 0.001$), and *NCOA2* ($p < 0.05$) significantly decreased after treatment with stigmasterol compared to the control group.

Discussion

Saw palmetto is recognized for its beneficial effects in treating BPH, yet the underlying molecular mechanisms remain largely undefined. In this study, we aimed to determine the

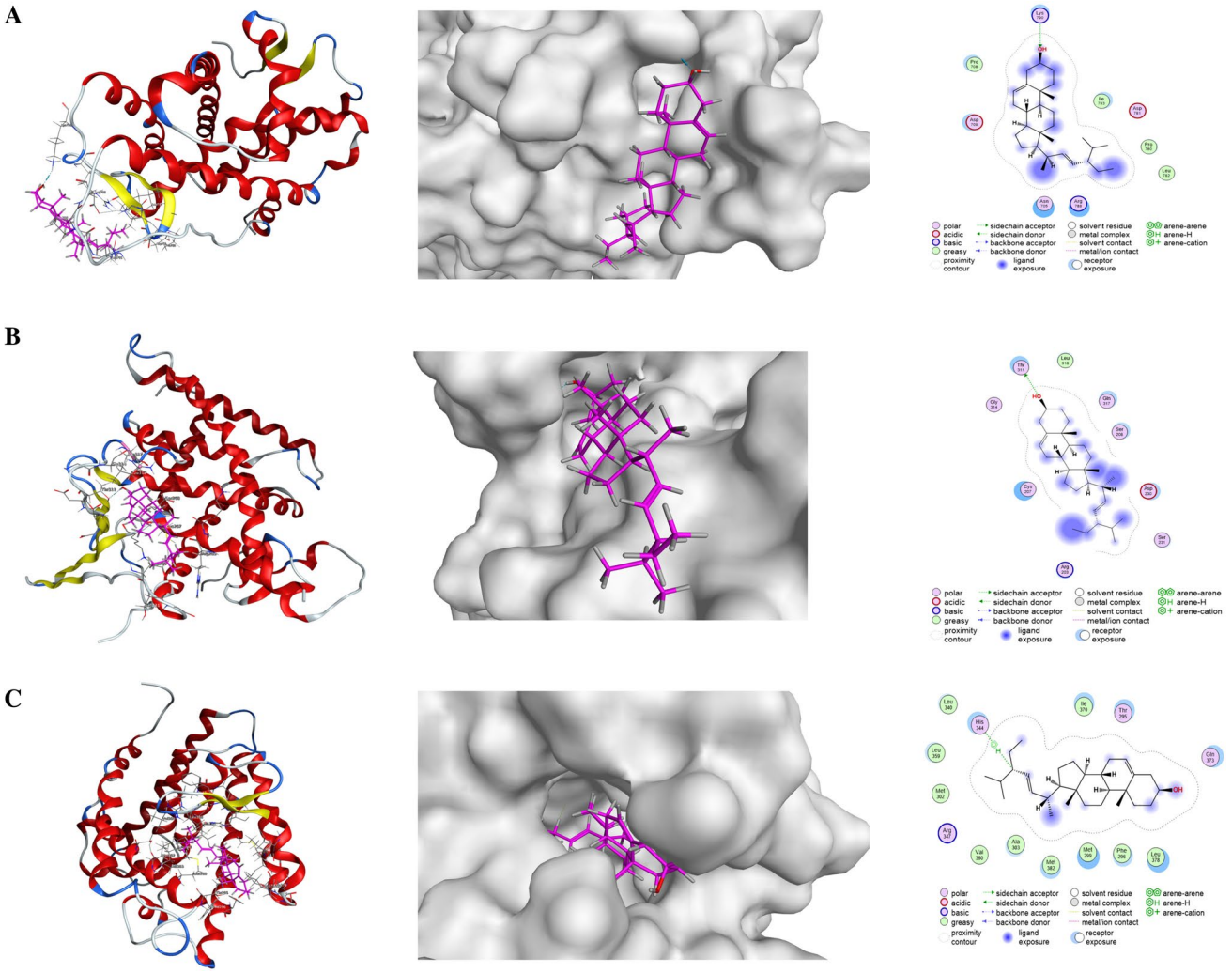

Fig. 5 Molecular docking patterns of stigmasterol with targets. **A** *PGR* protein-stigmasterol. **B** *NCOA1* protein-stigmasterol. **C** *NCOA2* protein-stigmasterol

potential therapeutic mechanism of saw palmetto in the treatment of BPH through network pharmacology and further validate this molecular mechanism through molecular docking and cellular experiments.

In this study, we identified 13 active components from saw palmetto. It is worth noting that stigmasterol, a key ingredient of saw palmetto, is found to be most closely associated with BPH-related targets through ingredient-target network analysis. This indicates that stigmasterol plays a pivotal role in the treatment of BPH. Stigmasterol, a phytosterol in saw palmetto, has anti-inflammatory and antioxidant pharmacological effects (Jie et al. 2022). Besides, stigmasterol showed significant biological activity in the regulation of cell function. For example, studies have indicated that stigmasterol can achieve anti-tumor effects by inhibiting the migration of gastric cancer cells, arresting cell cycle, promoting cell apoptosis, and blocking the JAK/STAT signaling pathway (Li et al. 2018). Lu et al. have revealed that stigmasterol inhibits the proliferation and promotes the apoptosis of fibroblast-like synovial cells through the PI3K/AKT signaling pathway, thereby having a positive effect on collagen-induced arthritis rat model (Lu et al. 2023). In addition, stigmasterol isolated from marine microalgae boat insert can induce apoptosis of human hepatocellular carcinoma HepG2 cells (Kim et al. 2014). These results show the potential application value of stigmasterol in treating human diseases.

We subsequently identified three key therapeutic targets (*PGR*, *NCOA1*, and *NCOA2*) that potentially mediate the protective effects of saw palmetto against BPH by integrating the PPI analysis (*PGR*, *NCOA1*, *NCOA2*, *ADRB1*, and *ADRB2* had the highest degree values), the KEGG results (the estrogen signaling pathway was the main enrichment pathway and was closely related to BPH (Liu et al. 2023; Liu et al. 2024; Fan et al. 2020)), and

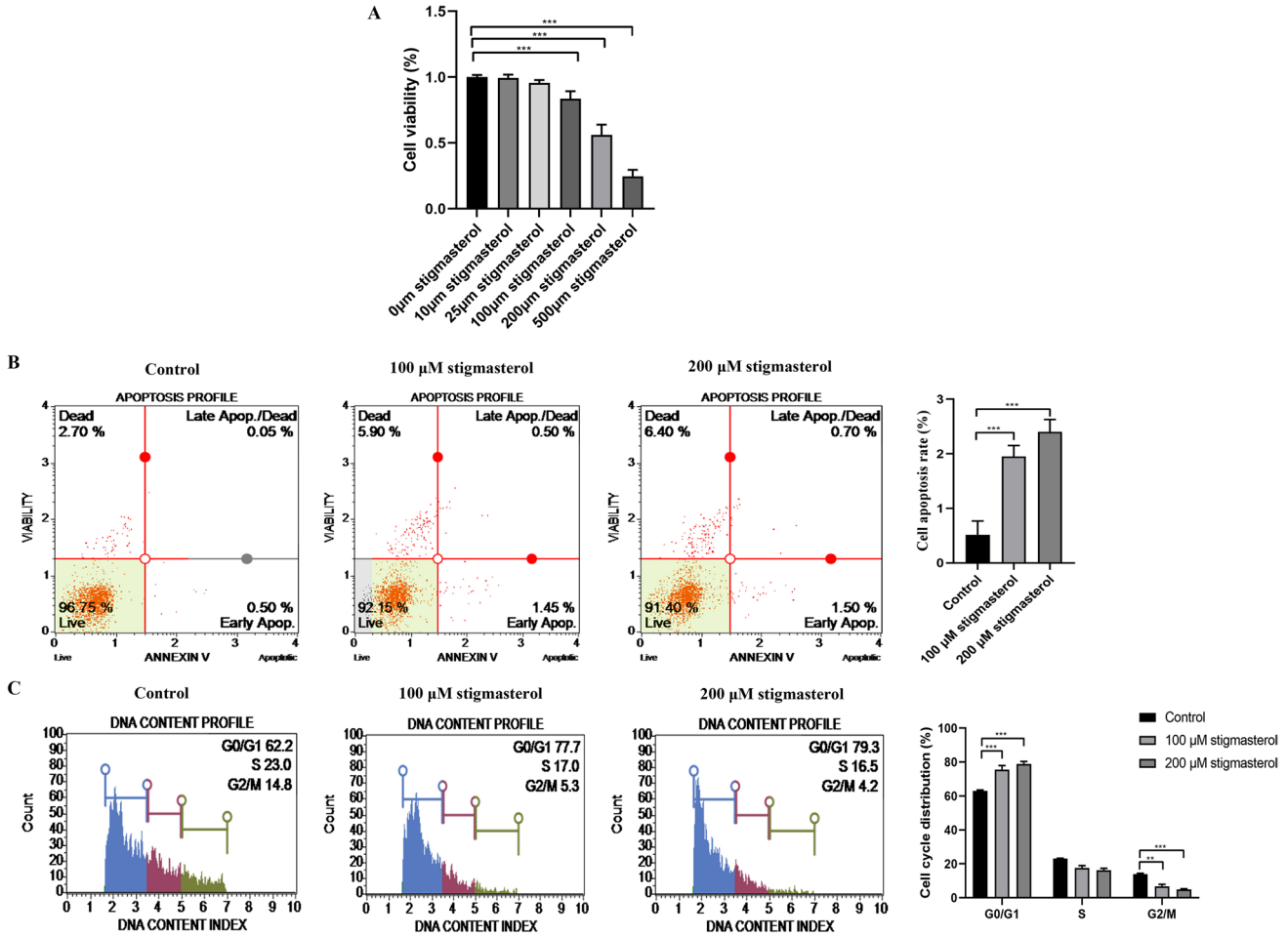

Fig. 6 Stigmasterol inhibits cell viability and division and promotes apoptosis of BPH-1 cells. Analysis of BPH-1 cell viability (**A**), apoptosis (**B**), and cell cycle (**C**) after stigmasterol treatment. All data was presented as mean ± standard deviation. $^{**}p < 0.01$, compared with the control group; $^{***}p < 0.001$, compared with the control group

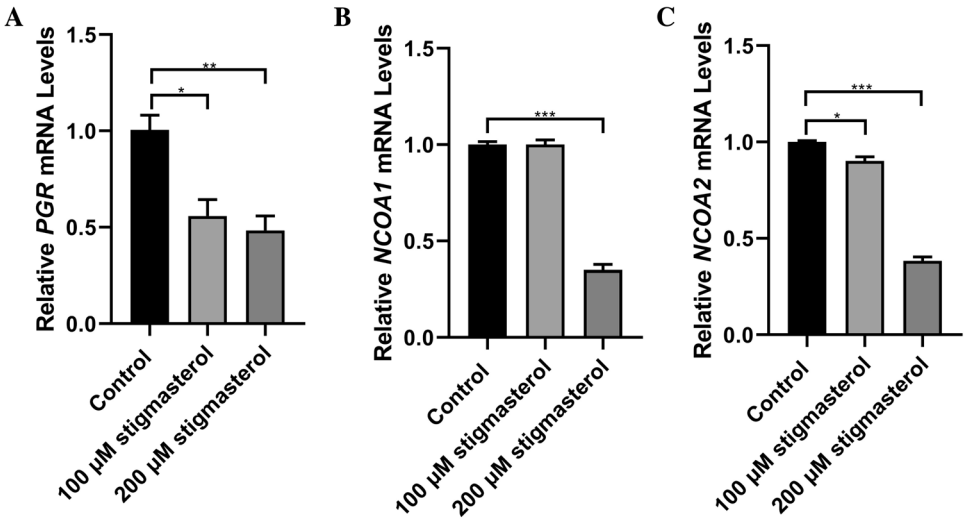

Fig. 7 Analysis of *PGR*, *NCOA1*, and *NCOA2* mRNA expression after stigmasterol treatment by qRT-PCR. **A** PGR, **B** *NCOA1*, and **C** *NCOA2* mRNA expression. All data was presented as mean ± standard deviation. $^{*}p < 0.05$, compared with the control group; $^{**}p < 0.01$, compared with the control group; $^{***}p < 0.001$, compared with the control group

the target-pathway network (demonstrating that *PGR*, *NCOA1*, and *NCOA2* participate in the estrogen signaling pathway). PGR, a nuclear receptor widely expressed in the reproductive system, including the prostate, regulates gene expression by binding to progesterone and then affects the biological functions of cells such as growth, differentiation, and apoptosis (Chen et al. 2017). The expression level of *PGR* is significantly upregulated in BPH, suggesting a possible promotional role in the pathogenesis of BPH (Song et al. 2016). In addition, research by Li et al. established a correlation between PGR genetic variants and the risk of developing BPH (Li and Klein 2021). *NCOA1* and *NCOA2* are a kind of protein that can enhance the signal transduction of nuclear receptors. By binding to nuclear receptors, they promote the transcription activation of target genes, thus influencing various biological processes, such as cell growth, differentiation, and metabolism. For example, a study revealed that the accumulation of *NCOA1*, without HERC3, can promote the degradation of the extracellular matrix, which may be related to the invasion and metastasis of prostate cancer (Li et al. 2023b). Besides, the mRNA expression level of *NCOA1* was found to be significantly decreased by treating with myrocin A in BPH (Khooblall et al. 2023; Kwon et al. 2024). Molecular docking analysis was further conducted to verify the interactions between a core component of saw palmetto and hub genes. Our study indicated strong binding affinities between stigmasterol and *PGR*, *NCOA1*, and *NCOA2*. These findings indicate that *PGR*, *NCOA1*, and *NCOA2* play an important role in saw palmetto against BPH.

Cellular experiments were subsequently carried out to verify whether stigmasterol modulated the expression of *PGR*, *NCOA1*, and *NCOA2*, thereby potentially playing a role in the treatment of BPH. First, the therapeutic effect of stigmasterol was detected by cell function experiments, including cell viability, apoptosis, and cycle. It was shown that stigmasterol could significantly increase the percentage of BPH-1 cells in the G0/G1 phase and inhibit cell viability and division. Second, the effect of stigmasterol on the mRNA expression of *PGR*, *NCOA1*, and *NCOA2* was evaluated by qRT-PCR analysis. We observed that stigmasterol could notably downregulate the expression of *PGR*, *NCOA1*, and *NCOA2*. Consistent with our results, Kang et al. indicated that phytosterol extracted from hull-less pumpkin can increase the expression of apoptosis-related factors in the BPH rat model (Kang et al. 2021). *Rauwolfia vomitoria* extract can significantly inhibit BPH epithelial cell viability by inducing apoptosis (Huang et al. 2022). Cheon et al. showed that oleanolic acid suppressed cell proliferation by reducing the expression of *PCNA* and cell cycle markers in the BPH animal model (Cheon et al. 2020). In addition, resveratrol suppressed the viability of WPMY-1 cells by inducing the G_0/G_1-phase cell cycle, which was a result of the downregulation of cyclin and cyclin-dependent kinase (Jang et al. 2021). Moreover, Wei et al. highlighted that cryptotanshinone may regulate the proliferation and apoptosis of BPH-1 cells by inhibiting the EGFR/STAT3 axis, thus inhibiting the progress of BPH (Wei et al. 2023). Taken together, we speculated that saw palmetto might influence the cell functions of BPH-1 cells by modulating the expression of *PGR*, *NCOA1*, and *NCOA2*, thereby playing a potential role in the treatment of BPH.

Our study has certain limitations. First, the current study did not determine the functions of *PGR*, *NCOA1*, and *NCOA2* in the treatment of BPH. Multi-level experiments are needed to further explore the specific mechanisms underlying saw palmetto's effects on these proteins in BPH treatment. Second, we have only verified the therapeutic effect of the active ingredients at the cellular level, and we will further verify it by constructing an animal model of BPH in the future. Third, the therapeutic mechanisms require further exploration and verification through in vivo experiments.

Conclusion

In this study, we integrated network pharmacology, molecular docking, and cellular experiments to explore the therapeutic mechanism of saw palmetto in BPH treatment. Our study indicated that saw palmetto, especially its stigmasterol component, might play a therapeutic role in BPH by suppressing the expression of *PGR*, *NCOA1*, and *NCOA2*, thereby inhibiting cell viability and division. This study enhances our understanding of TCM's therapeutic mechanisms and presents a promising strategy for establishing the scientific basis and treatment approach for TCM in disease management.

Authors contributions Bo Zhang conceptualized, wrote, and reviewed the manuscript. Yiying Wang contributed to the network pharmacology analysis. Kunping Yan conducted the experiments and data analysis. Jiangang Yang contributed to the experiments and interpretation. All authors contributed to the article and approved the submitted version. The authors declare that all data were generated in-house and that no paper mill was used.

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Declarations

Competing interests The authors declare no competing interests.

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