# Prediction and Validation of Multi-Target Mechanisms of *Scutellaria baicalensis* in Treating Primary Dysmenorrhea Based on Network Pharmacology and Molecular Docking

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**Abstract** [**Objectives**] To predict core targets and pathways of flavonoids from *Scutellaria baicalensis* against PD via network pharmacology. [**Methods**] Network pharmacology was employed to predict targets of six flavonoids (baicalein, baicalin, chrysin, wogonin, wogonoside, oroxylin A) from *S. baicalensis*. PD-related targets were screened from DrugBank, DisGeNET, GeneCards, and NCBI databases. Compound-target-disease networks and protein-protein interaction (PPI) networks were constructed. Functional enrichment analysis (GO/KEGG) was performed via Metascape. Molecular docking (Autodock Vina) validated ligand-target binding affinities. [**Results**] Intersection analysis identified 18 pivotal targets from 148 compound targets and 18 PD-associated targets. PPI network analysis revealed $PTGS_2$, $ESR_1$, TNF, and $ABCB_1$ as core targets (degree >6). KEGG enrichment highlighted ovarian steroidogenesis (hsa04913) and ABC transporters. Molecular docking confirmed robust binding between flavonoids and $PTGS_2$ (binding energy < −5 kcal/mol; baicalin: −13.2). [**Conclusions**] Flavonoids synergistically target $PTGS_2$/$ESR_1$-mediated prostaglandin synthesis and hormonal pathways.

**Key words** *Scutellaria baicalensis*, Primary dysmenorrhea (PD), Network pharmacology, $PTGS_2$, Molecular docking

## 1 Introduction

Primary dysmenorrhea (PD), a prevalent gynecological disorder, arises primarily from pathological uterine hypercontraction and localized ischemia-hypoxia. *Scutellaria baicalensis* Georgi, a traditional herb for clearing heat and drying dampness, demonstrates significant spasmolytic activity attributed to its flavonoid fraction. However, its precise active components and mechanisms remain incompletely characterized. Network pharmacology offers a systematic approach to analyze the multi-component, multi-target, multi-pathway therapeutic profile of botanical medicines. This study integrates network prediction and molecular docking validation to comprehensively delineate the active flavonoid ensemble of *S. baicalensis* and their synergistic mechanisms against PD, thereby furnishing a scientific foundation for its advanced development[1-3].

## 2 Materials and methods

**2.1 Compound target prediction** Canonical SDF structures of six flavonoids were retrieved from PubChem. SwissTargetPrediction (species: Homo sapiens) identified targets ($P>0$)[4], followed by deduplication.

**2.2 Disease target screening** PD-associated targets were mined from DrugBank[5], DisGeNET[6], GeneCards, and NCBI using "primary dysmenorrhea" as the query.

**2.3 Intersection targets and network construction** Venny 2.1 identified shared targets. Compound-target-disease networks were visualized in Cytoscape 3.8.0 (compounds: circular nodes; targets: rectangular nodes; degree-based layout).

**2.4 PPI network analysis** STRING-db (confidence >0.4) generated PPI networks. CytoNCA calculated topology parameters (Degree, Betweenness) to identify hub targets.

**2.5 Enrichment analysis** Metascape performed GO (Biological Process, Molecular Function, Cellular Component) and KEGG pathway enrichment. Results were visualized as bar/bubble plots.

**2.6 Molecular docking** Ligands (flavonoids) and receptor ($PTGS_2$, PDB: 5F1A) were prepared in Autodock Tools 1.5.6. Docking was executed via Autodock Vina; poses were rendered in PyMol.

## 3 Results and analysis

**3.1 Compound and disease targets** Targets of six flavonoids are summarized (Table 1). After deduplication, 148 unique compound targets were retained. Eighteen PD-associated targets were identified (Fig. 1), with TNF and $PTGS_2$ recurring across ≥3 databases[7-8].

**3.2 Compound-target-disease and PPI networks** Eighteen shared targets were identified (Fig. 2). Network topology analysis (Fig. 3) revealed $CA_2$, $PTGS_2$, and $ESR_1$ as highest-degree nodes. STRING analysis yielded 40 edges among 18 nodes (average degree = 4.44). Visualization in Cytoscape (Fig. 4) identified TNF, $ABCB_1$, $ESR_1$, and $PTGS_2$ as hub targets (Degree >6, Betweenness >11.866 6, Closeness >0.566 6). $PTGS_2$ and $ESR_1$ exhibited high centrality across networks[9-10].

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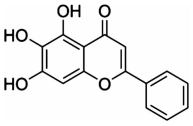
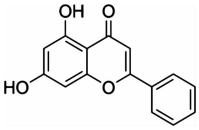
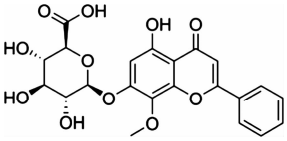
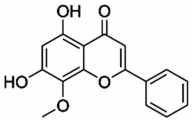
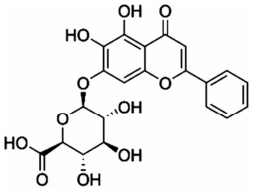
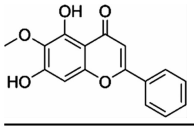
**Table 1 Active components in *Scutellaria baicalensis***

| Chemical structure | Name | Predicted targets (*n*) |
|---|---|---|
|  | Baicalein | 104 |
|  | Chrysin | 104 |
|  | Baicalin | 14 |
|  | Wogonin | 104 |
|  | Wogonoside | 19 |
|  | Oroxylin A | 104 |

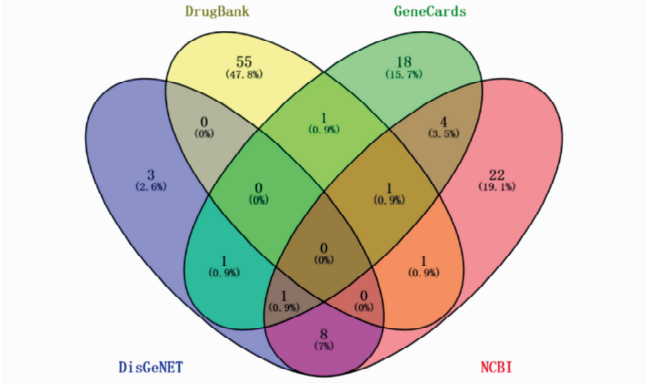



**Fig. 1 Venn diagram of disease targets**

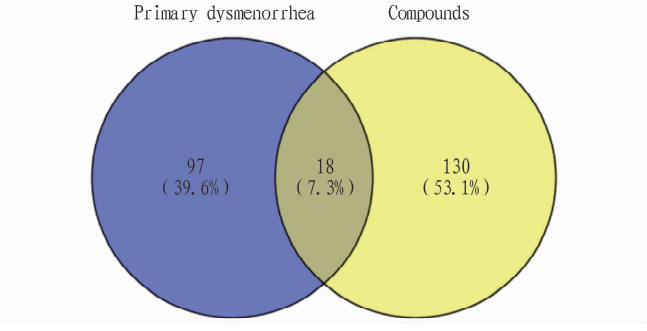



**Fig. 2 Compound-disease target intersection**

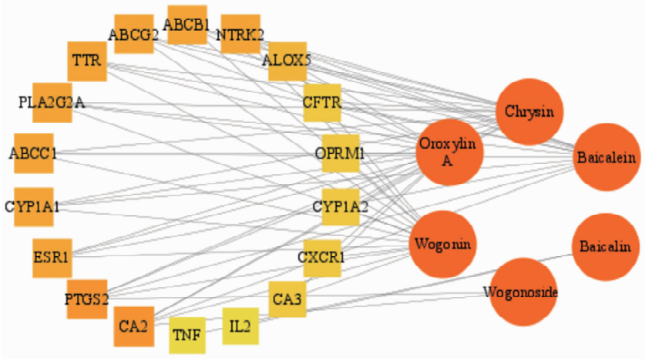



**Fig. 3 Compound-target-disease network**

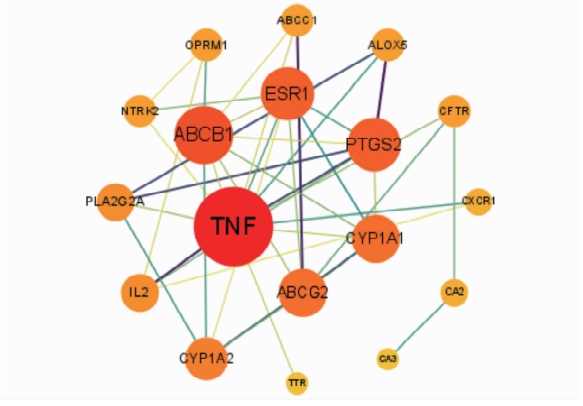



**Fig. 4 PPI network**

### 3.3 GO and KEGG enrichment analysis

**3.3.1** GO analysis (Fig. 5). Biological process: Regulation of inflammatory response, transepithelial transport, eicosanoid metabolic process, cellular response to organic nitrogen, chloride transport regulation. Cellular component: Apical plasma membrane, membrane raft, external side of plasma membrane, perinuclear cytoplasm, mitochondrial membrane. Molecular function: ABC-type transporter activity, hydrolase activity, heme binding, receptor-ligand activity, protein homodimerization.

**3.3.2** KEGG pathways. Significant enrichment in ABC transporters (hsa02010), antifolate resistance (hsa01524), ovarian steroidogenesis (hsa04913), and chemical carcinogenesis-DNA adducts (Fig. 6). $PTGS_2$-encoded $COX_2$ catalyzes prostaglandin synthesis in ovarian steroidogenesis[11] (Fig. 7) and antifolate resistance pathways (Fig. 8)[12].

### 3.4 Molecular docking[13]

All flavonoids exhibited strong binding to $PTGS_2$ (binding energy < −5 kcal/mol; Table 2). Baicalin demonstrated optimal affinity (−13.2 kcal/mol)[14]. Docking poses are illustrated in Fig. 9.

**Table 2 Molecular docking affinities**

| Component | Binding energy//kcal/mol |
|---|---|
| Baicalein | −10.5 |
| Chrysin | −10.8 |
| Baicalin | −13.2 |
| Wogonin | −9.4 |
| Wogonoside | −11.7 |
| Oroxylin A | −11.0 |

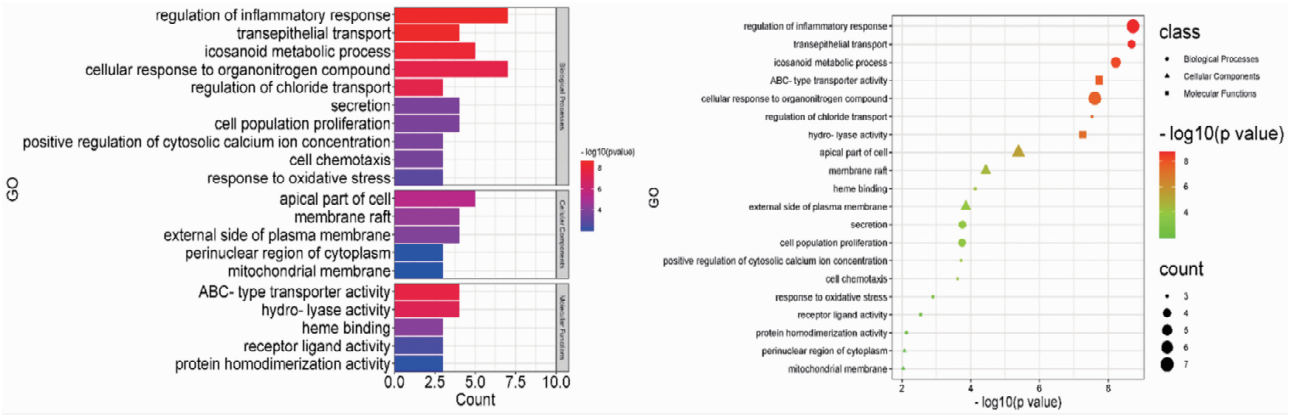



**Fig. 5 GO enrichment**

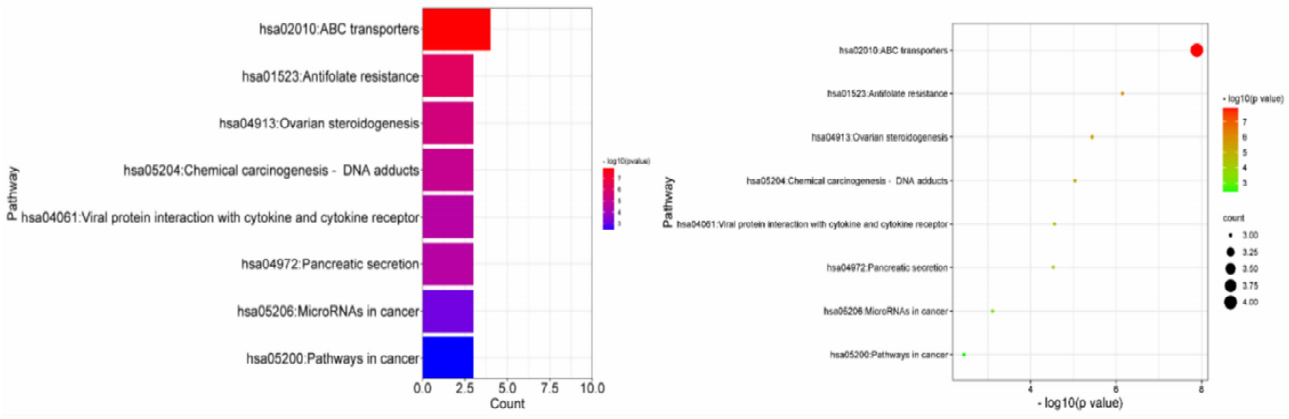



**Fig. 6 KEGG pathway enrichment**

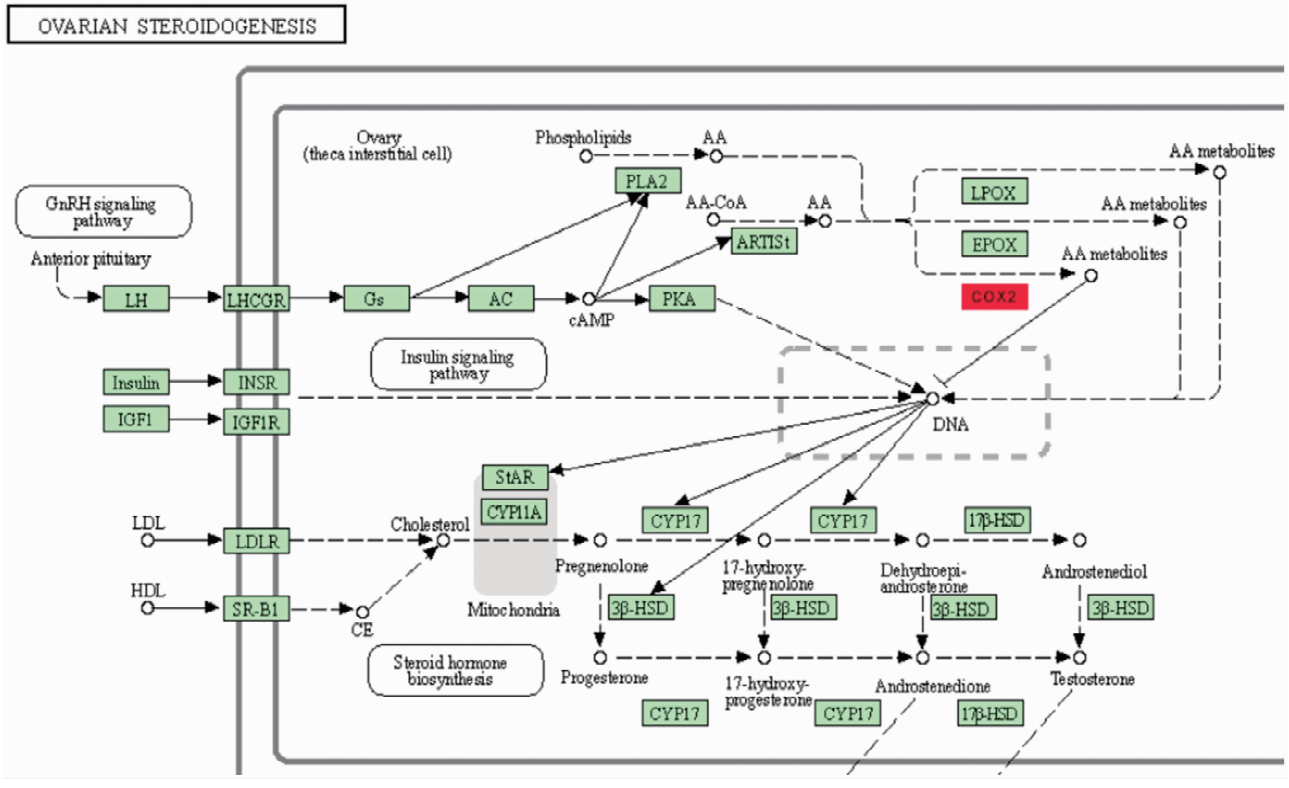



**Fig. 7 Ovarian Steroidogenesis Pathway**

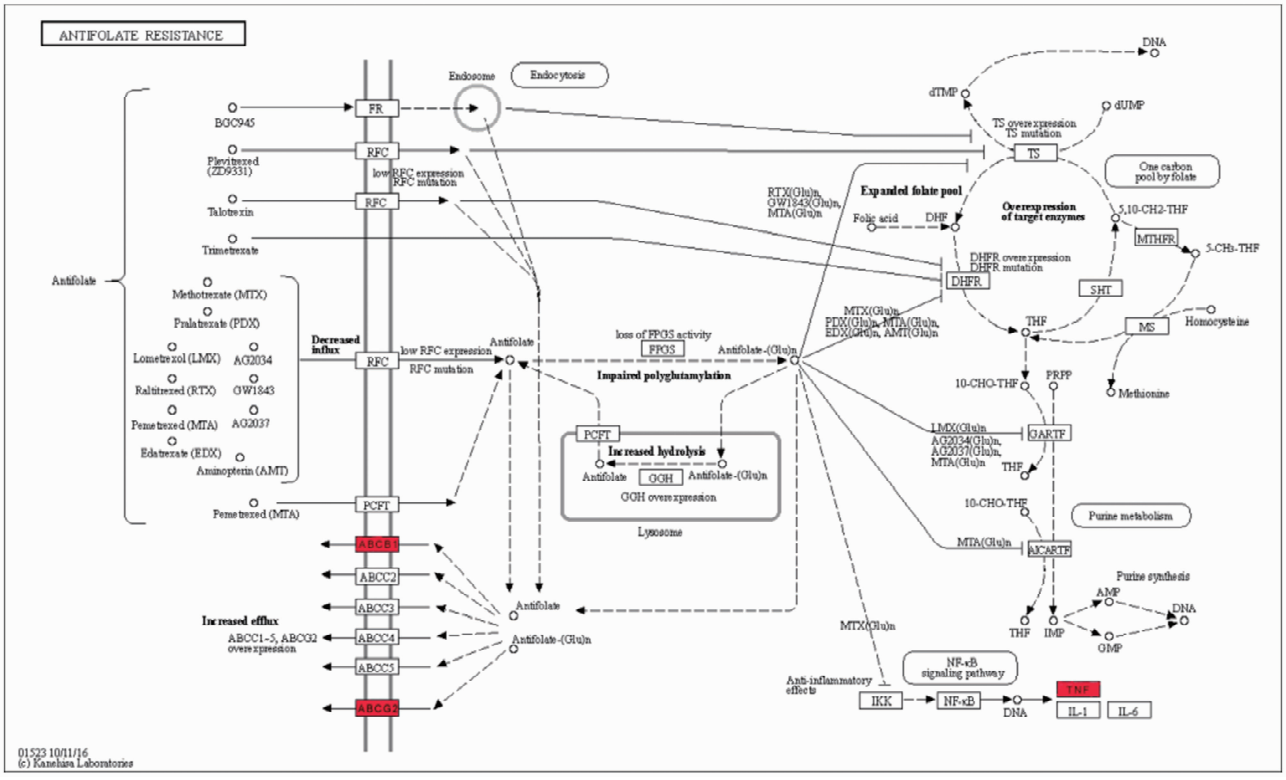



**Fig. 8 Antifolate resistance pathway**

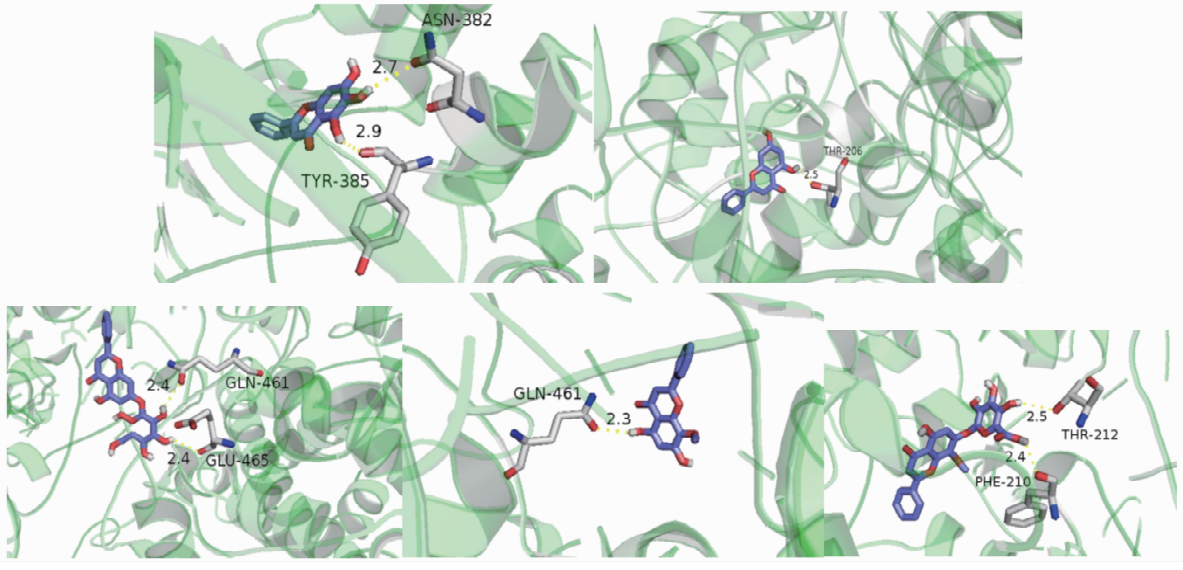



**Fig. 9 Molecular docking visualization**

## 4 Discussion

This study applies a systematic network pharmacology approach to elucidate the multi-target mechanisms of *S. baicalensis* flavonoids against PD. The identification of 18 pivotal targets at the compound-disease intersection, particularly $PTGS_2$ and $ESR_1$ as hub nodes in PPI networks, reveals a synergistic modulatory pattern. Three key insights emerge.

**4.1 $PTGS_2$ as the core therapeutic target** Molecular docking confirmed all six flavonoids bind robustly to $PTGS_2$ (binding energy $< -5$ kcal/mol), with baicalin exhibiting the highest affinity ($-13.2$ kcal/mol). This directly suppresses COX-2 enzymatic activity, reducing PGF-α synthesis, a principal mediator of uterine hypercontraction and ischemia in PD[14-15].

**4.2 Multi-pathway synergy** KEGG enrichment highlights ovarian steroidogenesis (hsa04913) and ABC transporters (hsa02010) as dominant pathways. $PTGS_2$-mediated prostaglandin dysregulation intersects with $ESR_1$-driven hormonal responses, ex-

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guarding the quality and safety of Jiulongteng honey. Furthermore, they contribute to the enhancement of regulatory measures within the honey market, protect the legitimate rights and interests of consumers, and promote the sustainable development of the Jiulongteng honey industry.

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plaining traditional "Heat-Clearing" efficacy of *S. baicalensi*[1,11].

**4.3 Flavonoid ensemble advantage** Despite structural similarities, baicalin (lower predicted targets) showed strongest $PTGS_2$ binding, while baicalein/wogonin modulate broader targets (such as TNF, $ABCB_1$). This suggests functional complementarity-where baicalin potently inhibits prostaglandin synthesis, other flavonoids concurrently regulate inflammation and calcium signaling[7,12].

## 5 Conclusions

*S. baicalensis* alleviates PD through a flavonoid ensemble (baicalein, baicalin, wogonin, *etc.*) that synergistically targets $PTGS_2$-mediated prostaglandin synthesis, $ESR_1$-linked hormonal regulation, and calcium signaling pathways. Network pharmacology and molecular docking validate: $PTGS_2$ inhibition as the central mechanism, with baicalin exhibiting optimal binding affinity. Co-regulation of ovarian steroidogenesis and ABC transporters plays as key therapeutic pathways. These findings will provide a mechanistic foundation for developing *S. baicalensis* based multi-target therapeutics against PD.

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