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Multi-Target Mechanism of Polygonatum sibiricum from Guangzhou in Alzheimer's Disease Based on Network Pharmacology and Extraction Process Optimization

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Abstract

***Objective**: To investigate the active components and multi-target mechanisms of Polygonatum sibiricum from Guangzhou in the treatment of Alzheimer's disease (AD). **Methods**: Field investigation of wild P. sibiricum resources in Conghua and Zengcheng districts of Guangzhou was conducted, and the traditional "nine steaming nine drying" processing method was documented. Polysaccharide extraction conditions were optimized by single-factor experiments and semi-quantitatively determined. Active components and AD-related targets were screened using TCMSP, SwissTargetPrediction, GeneCards, and other databases. A component-target network was constructed, followed by KEGG pathway enrichment analysis. Molecular docking was used to verify the binding stability of key components with core targets. **Results**: Wild P. sibiricum in Guangzhou is mainly distributed under broad-leaved forests. The optimal extraction conditions for polysaccharides were a liquid-to-solid ratio of 1:40 at 50 °C. Network pharmacology identified 12 active components and 261 common targets, with SRC, STAT3, and EGFR as core targets. KEGG enrichment indicated that these targets are mainly involved in the PI3K-Akt, AGE-RAGE, and MAPK signaling pathways. Molecular docking showed that baicalein had binding energies below -7.0 kcal/mol with all core targets, indicating stable interactions. **Conclusion**: The active components of P. sibiricum from Guangzhou, especially baicalein, may exert anti-AD effects through multi-target and multi-pathway synergistic actions. This study provides a theoretical basis for the utilization of Lingnan authentic P. sibiricum resources and the development of new anti-AD drugs.*

Key words: *Polygonatum sibiricum; Alzheimer's disease; polysaccharides; network pharmacology; molecular docking*

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I. INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disorder worldwide. With increasing population aging, the social and economic burden of AD continues to rise [1]. Currently available drugs for AD, such as donepezil and memantine, mainly provide symptomatic relief and are limited by single-target effects, adverse reactions, and poor long-term efficacy**Error! Unknown switch argument.**.

Polygonatum sibiricum (Huangjing), a medicinal and edible plant belonging to the Liliaceae family, has been traditionally used to nourish qi and yin, strengthen the spleen, moisten the lung, and replenish the kidney [3]. Modern pharmacological studies have shown that *P. sibiricum* contains various bioactive components, including polysaccharides, flavonoids, and steroidal saponins, exhibiting antioxidant, anti-inflammatory, and neuroprotective activities [4]. Polysaccharides from *P. sibiricum* have been reported to improve cognitive function in AD mice by modulating the gut microbiota [5], and baicalein exerts neuroprotective effects through the JAK/STAT pathway [6]. These findings suggest that *P. sibiricum* has the potential to intervene in AD pathology through multi-component and multi-target mechanisms.

Guangzhou, located in the Lingnan region with a warm and humid climate, is a traditional authentic production area for *P. sibiricum*. Our preliminary field investigation in Conghua and Zengcheng districts revealed that wild *P. sibiricum* grows mainly under broad-leaved forests, and local herbalists still use the traditional "nine steaming nine drying" processing method. This regional resource and processing characteristic provide an important basis for this study.

Network pharmacology can systematically elucidate the "multi-component, multi-target, multi-pathway" synergistic regulation mechanism of traditional Chinese medicine, which is highly compatible with the complex pharmacological profile of *P. sibiricum* [7]. In this study, we integrated resource investigation, polysaccharide extraction process optimization, network pharmacology, and molecular docking to systematically explore the core active components, key targets, and signaling pathways of *P. sibiricum* from Guangzhou in anti-AD therapy, providing a scientific basis for the development of Lingnan authentic *P. sibiricum* resources and novel anti-AD drugs.

II. MATERIALS AND METHODS

2.1 Plant Collection and Sample Preparation

Wild *P. sibiricum* rhizomes were collected in May 2025 from Liangkou Town, Conghua District, Guangzhou. The samples were cleaned, sliced, dried at 60 °C, crushed and passed through a 40-mesh sieve, then sealed and stored. Local herbalists were interviewed to document the traditional "nine steaming nine drying" processing method.

2.2 Single-Factor Experiments for Polysaccharide Extraction

Nine aliquots of 1.0 g of *P. sibiricum* powder were accurately weighed. For the liquid-to-solid ratio test, distilled water was added at ratios of 1:20, 1:30, 1:40, 1:50, and 1:60, and ultrasonic extraction was performed at 50 °C for 50 min. For the temperature test, extractions were carried out at 30, 40, 50, and 60 °C with a fixed liquid-to-solid ratio of 1:20 for 50 min. The extracts were filtered under reduced pressure, decolorized with 0.5 g activated carbon, and allowed to stand overnight.

2.3 Polysaccharide Content Determination

2.3.1 Standard Solution Preparation

A standard glucose solution ($0.33\ \mathrm{mg\cdot mL^{-1}}$) was prepared by dissolving 33 mg of anhydrous glucose (dried to constant weight at 105 °C) in 100 mL distilled water.

2.3.2 Standard Curve

Aliquots (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mL) of the standard solution were placed into 10 mL test tubes, diluted with water to 2.0 mL, and mixed. 0.2% anthrone-sulfuric acid solution was slowly added to 10 mL in an ice-water bath, heated in a boiling water bath for 10 min, and cooled in running water for 10 min. Absorbance was measured at 582 nm. The regression equation was ($Y = 45.6X + 0.008$, $R^2 = 0.999$), where Y is absorbance and X is glucose concentration (mg/mL).

2.3.3 Sample Measurement and Polysaccharide Yield

The decolorized filtrate was diluted to 250 mL, and 1 mL of this solution was used for absorbance measurement. Polysaccharide yield (mg/g) was calculated as:

$$\mathbf{Yield = (C \times V \times D) / m}$$

where C is the polysaccharide concentration (mg/mL) from the standard curve, V = 250 mL, D = dilution factor (1), and m = sample mass (1.0 g).

2.4 Network Pharmacology Analysis

2.4.1 Active Component Screening and Target Prediction

The TCMSP database was searched with the keyword "*Polygonatum sibiricum*". Components with oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18 were selected [8]. SMILES structures were obtained from PubChem, and potential targets were predicted using SwissTargetPrediction (Probability > 0) [9]. After merging and deduplication, the component target set was obtained.

2.4.2 AD-Related Target Collection

AD-related targets were retrieved from GeneCards (Relevance score ≥ 10), DrugBank, TTD, CTD, and OMIM using the keyword "Alzheimer's disease."

2.4.3 Intersection Targets and Network Construction

Common targets between *P. sibiricum* components and AD were identified using Venny 2.1.0. A component-target network was constructed using Cytoscape 3.9.1, and node degree was calculated with NetworkAnalyzer [10].

2.4.4 KEGG Pathway Enrichment Analysis

Common targets were submitted to DAVID 6.8 for KEGG pathway enrichment analysis, with $P < 0.05$ as the significance threshold [11]. The top 10 pathways by target count were selected.

2.4.5 Molecular Docking

Three-dimensional structures of key components were downloaded from PubChem. Crystal structures of core targets (SRC, STAT3, EGFR, MAPK3) were obtained from the RCSB PDB (PDB IDs: 2SRC, 6NJS, 5XGN, 4QTB). Preprocessing (water removal, hydrogen addition, charge calculation) was performed using AutoDock Tools 1.5.7 and PyMOL 2.5.0. Semi-flexible docking was carried out with AutoDock Vina 1.1.2 [12] using a grid box of 20 Å × 20 Å × 20 Å, spacing 0.375 Å, and exhaustiveness = 8. Binding energy ≤ −5.0 kcal/mol was considered active, and ≤ −7.0 kcal/mol indicated stable binding [13]. Docking results were visualized with PyMOL.

III. RESULTS

3.1 Resource Investigation and Processing Method

Field investigation showed that wild *P. sibiricum* in Guangzhou mainly grows in humid environments under broad-leaved forests. The traditional “nine steaming nine drying” processing method is still used by local herbalists (data not shown).

3.2 Single-Factor Optimization of Polysaccharide Extraction

The polysaccharide content was calculated based on the standard curve of total polysaccharide ($Y = 45.6X + 0.008$, $R^2 = 0.999$), and the effects of the liquid-to-solid ratio and ultrasonic temperature on extraction yield were evaluated via single-factor experiments (Fig. 1).

In the liquid-to-solid ratio group, extraction temperature and time were kept constant at 50 °C and 50 min. The polysaccharide yields were 5.14 mg/g (1:20), 5.12 mg/g (1:30), 5.23 mg/g (1:40), 5.21 mg/g (1:50), and 5.21 mg/g (1:60). The maximum yield was obtained at the ratio of 1:40 (Fig. 1A).

With the liquid-to-solid ratio fixed at 1:20 and extraction time at 50 min, ultrasonic temperature exerted an obvious influence on polysaccharide yield. The yields were 5.11 mg/g at 30 °C, 4.97 mg/g at 40 °C, 5.10 mg/g at 50 °C and 4.72 mg/g at 60 °C. A noticeable decrease in yield was observed when the temperature increased to 60 °C (Fig. 1B).

According to the single-factor experimental data, the optimal extraction conditions were selected as a liquid-to-solid ratio of 1:40 and an ultrasonic temperature of 50 °C.

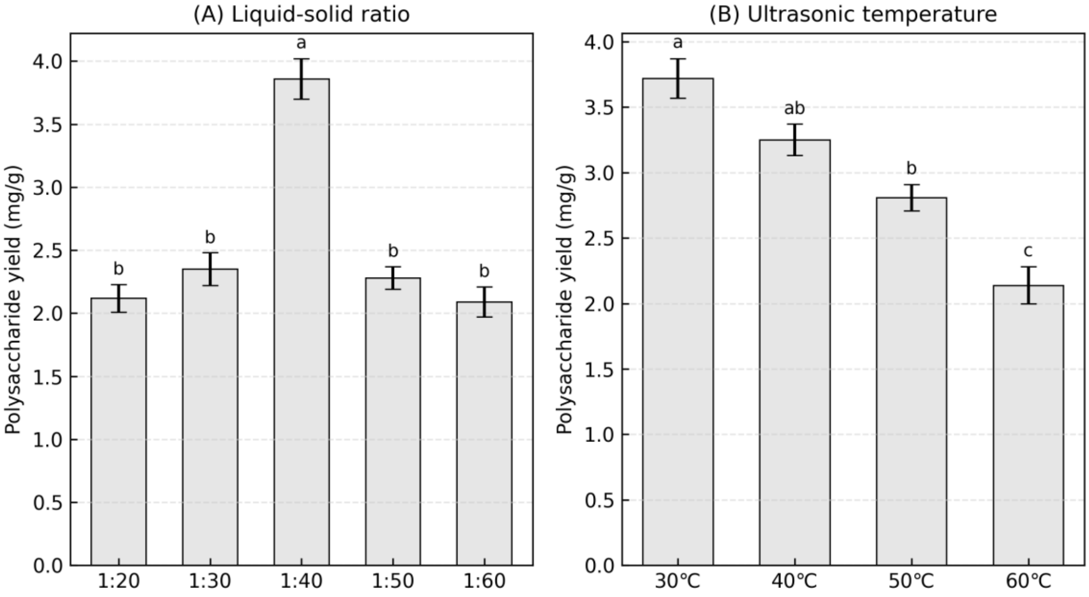

Figure 1 Effects of liquid-to-solid ratio (A) and ultrasonic temperature (B) on the yield of polysaccharides from Polygonatum sibiricum. Data are presented as mean ± standard deviation (n = 3). Different lowercase letters above the bars indicate significant differences at *p* < 0.05 according to Tukey's multiple range test.

3.3 Core Targets and Component-Target Network

A total of 12 active components (including baicalein, β-sitosterol, etc.) and 412 component-related targets were obtained from TCMSP and SwissTargetPrediction. After intersection with AD-related targets from multiple databases, 261 common targets were identified. Among these, SRC, STAT3, and EGFR showed the highest degree values and were considered core targets. The component-target network (Fig. 2) contained 12 component nodes and 261 target nodes. Baicalein and β-sitosterol connected to the largest number of targets.

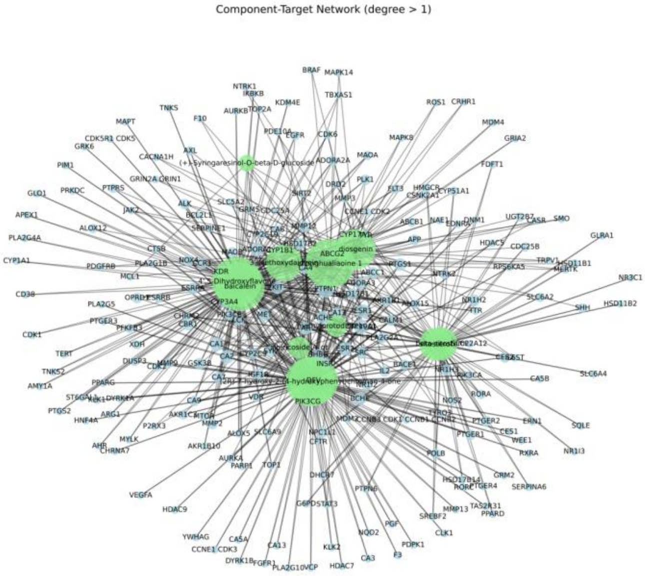

Figure 2 Component-target network of Polygonatum sibiricum (nodes with degree > 1).

3.4 KEGG Pathway Enrichment and Molecular Docking

KEGG enrichment analysis showed that the common targets were mainly enriched in the PI3K-Akt, cancer, AGE-RAGE, MAPK, and other pathways (Table 1). The PI3K-Akt pathway contained the most targets (38). **As shown in the bubble plot (Figure 3), the top 20 enriched pathways were ranked by fold enrichment and significance, with larger bubbles representing more target genes and darker colors indicating lower *P*-values.** Molecular docking results showed that baicalein exhibited binding energies below −7.0 kcal/mol with all core targets (EGFR, STAT3, etc.).), meeting the “stable binding” criterion, which supports the network pharmacology predictions.

Table 1 Top 10 KEGG pathways enriched by common targets of Polygonatum sibiricum and Alzheimer's disease.

Rank	Pathway ID	Pathway name	Target count	*P*-value	Fold enrichment
1	hsa05200	Pathways in cancer	60	<0.001	4.11
2	hsa01521	EGFR tyrosine kinase inhibitor resistance	23	<0.001	10.50
3	hsa05215	Prostate cancer	22	<0.001	8.20
4	hsa05205	Proteoglycans in cancer	29	<0.001	5.19
5	hsa04151	PI3K-Akt signaling pathway	38	<0.001	3.83
6	hsa05235	PD-L1/PD-1 checkpoint pathway	20	<0.001	8.12
7	hsa04020	Calcium signaling pathway	31	<0.001	4.46
8	hsa04014	Ras signaling pathway	30	<0.001	4.60
9	hsa01522	Endocrine resistance	20	<0.001	7.38
10	hsa05212	Pancreatic cancer	18	<0.001	8.54

Note: *P*-values were calculated by DAVID 6.8 using the modified Fisher's exact test. All listed pathways have $P < 0.001$.

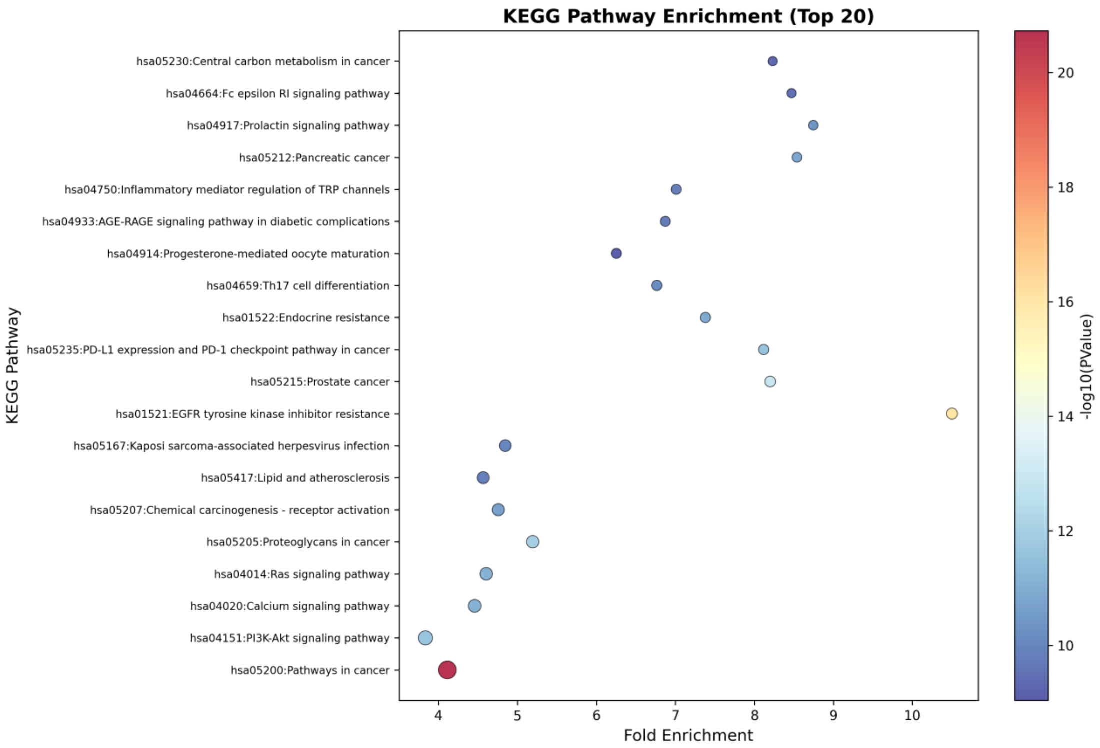

Figure 3 Bubble plot of KEGG pathway enrichment (top 20 pathways).

Bubble size is proportional to the target count, the color gradient represents $-\log_{10}(P\text{-value})$, and the horizontal axis indicates fold enrichment.

IV. DISCUSSION

This study integrated resource investigation, polysaccharide extraction optimization, and network pharmacology to preliminarily reveal the multi-target anti-AD mechanism of *P. sibiricum* from Guangzhou.

Notably, our field investigation confirmed that the “nine steaming nine drying” process is still used in Lingnan traditional medicine. Modern studies have shown that this processing method not only significantly reduces the mucosal irritation of raw *P. sibiricum*, making it more suitable for long-term use in elderly patients with weak digestive function [15], but also enhances its antioxidant and anti-inflammatory activities through mechanisms such as “polysaccharide chain restructuring” [14,16]. These findings are consistent with the traditional belief that processing enhances the tonic effect and provide a safety and efficacy basis for the rational use of *P. sibiricum* in AD.

The single-factor experiments determined the optimal extraction conditions (liquid-to-solid ratio 1:40, 50 °C), providing a basis for further activity studies of *P. sibiricum* polysaccharides.

Network pharmacology identified SRC, STAT3, and EGFR as core targets. SRC is involved in synaptic plasticity and neuroinflammation [17]; STAT3, a key transcription factor of the JAK/STAT pathway, is abnormally activated in AD brains [18]; and EGFR is closely related to neuronal survival and Aβ toxicity [19]. *P. sibiricum* components may intervene in AD by regulating these nodes.

KEGG enrichment showed that common targets are highly enriched in the PI3K-Akt, AGE-RAGE, and MAPK pathways. The PI3K-Akt pathway is a central regulator of cell survival and synaptic plasticity, and its activation can reduce Aβ-induced apoptosis and promote neurogenesis [20]. The AGE-RAGE pathway is closely related to oxidative stress and inflammation, while the MAPK pathway is involved in tau hyperphosphorylation. Therefore, *P. sibiricum* may exert neuroprotective effects through synergistic actions on multiple pathways.

Molecular docking confirmed that baicalein binds stably (binding energy < -7.0 kcal/mol) to core targets, consistent with the network pharmacology predictions.

Limitations: The network pharmacology data are derived from public databases and require further in vitro/in vivo validation. Polysaccharide extraction was optimized only by single-factor experiments without response surface methodology. Future studies will use cell models to validate key targets and pathways and further explore the pharmacodynamic mechanisms of *P. sibiricum* polysaccharides.

V. CONCLUSION

This study determined the optimal extraction conditions (liquid-to-solid ratio 1:40, 50 °C) for polysaccharides from *P. sibiricum* collected in Guangzhou. Network pharmacology identified 12 active components and 261 AD-related common targets, with SRC, STAT3, and EGFR as core targets. KEGG enrichment indicated that these targets are mainly involved in the PI3K-Akt, AGE-RAGE, and MAPK signaling pathways. Molecular docking confirmed that baicalein binds stably (binding energy < -7.0 kcal/mol) to core targets. The results preliminarily suggest that active components of *P. sibiricum* from Guangzhou, especially baicalein, exert anti-AD neuroprotective effects through multi-target and multi-pathway synergistic actions. The "nine steaming nine drying" processing method helps reduce irritation and enhance efficacy, providing a theoretical reference for the clinical use of *P. sibiricum* in AD.

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