Research Paper

Integrating Bioinformatics and Experimental Data to Validate the Mechanisms by which Active Components of *Astragalus* Regulate Neurotransmitter Metabolism in Parkinson's disease

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Zhang *et al*.: Mechanism of Astragalus *membranaceus* in the Treatment of Parkinson's disease

This study aims to explore the potential mechanisms of *Astragalus membranaceus* in the treatment of Parkinson's disease, with a particular focus on its role in neurotransmitter regulation. Firstly, using network pharmacology methods, we screened potential active ingredients from *Astragalus membranaceus* based on traditional Chinese medicine systems pharmacology database criteria, with oral bioavailability ≥40 % and drug-likeness ≥0.1. Subsequently, potential protein targets of these active ingredients were predicted using databases such as SwissTargetPrediction and Stitch. Targets related to Parkinson's disease and dopamine metabolism were obtained from GeneCards and Online Mendelian Inheritance in Man databases. We then constructed networks of active ingredients and targets, further filtering out targets associated with Parkinson's disease. Lastly, a protein-protein interaction network was constructed using the STRING database, and key genes in the network were quantified using the MCODE application. Functional enrichment analysis was performed using gene ontology/Kyoto encyclopedia of genes and genomes. Finally, molecular docking was employed to validate target genes. Our findings identified the CHRM2 gene as one of the potential targets of *Astragalus membranaceus* in treating Parkinson's disease. Further bioinformatics analysis revealed the modulatory effect of *Astragalus membranaceus* on the acetylcholine receptor signaling pathway, providing new theoretical insights into its neuroprotective effect. This study, employing a comprehensive approach of network pharmacology and bioinformatics analysis, elucidated the potential mechanisms of *Astragalus membranaceus* in Parkinson's disease treatment, emphasizing the significant role of neurotransmitter regulation.

Key words: *Astragalus membranaceus*, Parkinson's disease, network pharmacology, bioinformatics, neurotransmitter regulation

Parkinson's Disease (PD) is a chronic and progressive neurodegenerative disorder predominantly affecting elderly individuals[1]. A key pathological characteristic of PD is the substantial degeneration of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc) of the midbrain[2-4]. Normally, these neurons release dopamine to regulate motor functions in the striatum. When these neurons are lost, it leads to a marked reduction in dopamine levels in the striatum[5]. Another pathological characteristic of PD is the formation of Lewy bodies, which are mainly composed of aggregated alpha-synuclein and are present in the remaining dopaminergic neurons[6]. PD is also associated with significant neuroinflammation, characterized by the activation of microglia and the increased release of pro-inflammatory cytokines[7-9].

Dopamine is a crucial neurotransmitter that regulates motor, emotional, and cognitive functions[10]. In PD patients, the loss of dopaminergic neurons results in a significant reduction in central nervous system dopamine levels, leading to primary symptoms such as tremors, rigidity, bradykinesia, and postural instability[11]. Additionally, PD patients may experience non-motor symptoms, including depression, anxiety, sleep disturbances, and autonomic dysfunction[12].

Scutellaria baicalensis (*S. baicalensis*), commonly known as *Astragalus*, is a traditional Chinese medicinal herb widely used for treating inflammation, infections, and neurological disorders[13]. Its primary active components include baicalin, baicalein, wogonoside, and wogonin[14,15]. Modern research has demonstrated that *S. baicalensis* and its main constituents have significant neuroprotective

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effects[16]. Baicalin and baicalein possess potent anti-inflammatory properties, capable of inhibiting microglial activation and reducing the release of pro-inflammatory cytokines, thereby alleviating neuroinflammation[17,18]. These compounds can also scavenge free radicals and reduce oxidative stress, thus protecting dopaminergic neurons from damage[19]. Furthermore, baicalin regulates the expression of apoptosis-related genes, inhibiting neuronal apoptosis and slowing the progression of neurodegenerative diseases. Additionally, the components of *S. baicalensis* can promote the expression of nerve growth factors and synaptic formation, aiding in the recovery of neurological functions.

This study aims to systematically identify and validate the mechanisms by which *S. baicalensis* and its major components regulate dopamine neurotransmitter metabolism in PD. Network pharmacology methods will be used to predict the potential targets of the main components of *S. baicalensis*, especially those related to dopamine metabolism. Pathway enrichment analysis will be conducted on the predicted targets to identify the key pathways involved in dopamine metabolism.

MATERIALS AND METHODS

Network pharmacology analysis:

The chemical structures of the major active components of *S. baicalensis* (baicalin, baicalein, wogonoside, and wogonin) were sourced from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (http://tcmspw.com/tcmsp.php).

Target prediction:

Utilize databases such as SwissTarget (http://www.SwisstargetPrediction.ch/) Prediction and Stitch to predict potential protein targets of the identified active compounds.

PD-related target screening:

Utilize general databases from Online Mendelian Inheritance in Man (OMIM) (https://omim.org/), and GeneCards (https://www.genecards.org/) to search for genes and proteins linked to PD.

Protein-Protein Interaction (PPI):

Common targets were entered into the Search Tool for the Retrieval of Interacting Genes (STRING) database (https://string-db.org/) for analysis. The protein type was set to *Homo sapiens*, and the minimum interaction threshold was set to 0.4.

Network construction:

Construct a compound-target network using Cytoscape version 3.8.2 to visualize the interactions between the active compounds and their predicted targets.

Pathway enrichment analysis:

Perform pathway enrichment analysis on the identified targets using tools such as DAVID or the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database to identify key pathways related to dopamine metabolism. Focus on pathways known to be involved in the pathophysiology of PD.

Molecular docking:

Obtaining the SDF format files of core drug's major active ingredients from the PubChem database, collecting critical target protein structures from the Protein Data Bank (PDB) database, optimizing the targets using Pymol software by removing water molecules and small molecule ligands, and performing hydrogenation and charge processing using AutoDock Tools, then saving them as pdbqt format.

RESULTS AND DISCUSSION

To further explore the molecular mechanisms of *Astragalus* in combating PD, we conducted a network pharmacology and molecular docking study. We identified potential active compounds in *Astragalus* based on screening criteria of Oral Bioavailability (OB) ≥40 % and Drug-Likeness (DL) ≥0.1. Ultimately, we identified 27 potential active compounds from *Astragalus* (Table 1).

We utilized databases such as SwissTargetPrediction and Stitch to predict potential protein targets of active ingredients in *Astragalus membranaceus* (*A. membranaceus*). Subsequent analysis yielded a substantial number of potential targets. Simultaneously, through searches in GeneCards and OMIM databases using keywords PD and dopamine metabolism, we obtained a set of targets relevant to these diseases.

Using the mapping tool of Venny 2.1 online software, we compared the predicted targets with disease-associated targets (fig. 1A), thereby selecting specific targets closely related to PD and dopamine metabolism.

We illustrated the relationships between active ingredients of *A. membranaceus* and their targets through compound-target networks and target-disease networks (fig. 1B). Furthermore, we delineated the positions and roles of these targets in PD-related pathways.

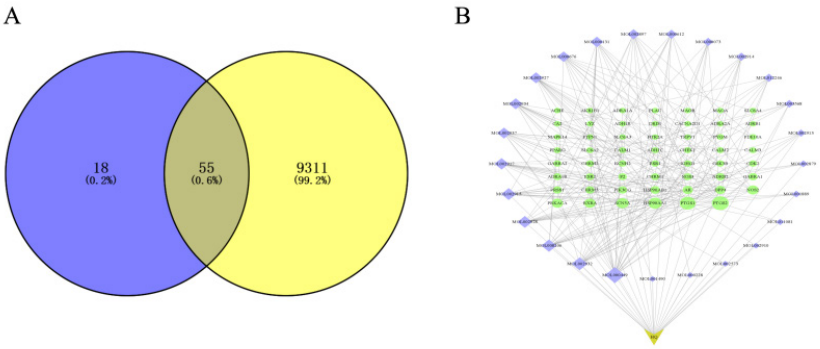

Fig. 1: Overlapping target genes and the drug-compound-target-disease network between *Astragalus* and PD, (A): Venn diagram showing the common drug-disease targets between *Astragalus* and PD and (B): Overlapping target genes and the drug-compound-target-disease network between *Astragalus* and PD

TABLE 1: INFORMATION ON ACTIVE INGREDIENTS OF *A. membranaceus*

ID	Molecule	OB (%)	DL
MOL000228	(2R)-7-hydroxy-5-methoxy-2-phenylchroman-4-one	55.23	0.2
MOL002573	ß-patchoulene	50.69	0.11
MOL002910	Carthamidin	41.15	0.24
MOL002911	2,6,2',4'-tetrahydroxy-6'-methoxychaleone	69.04	0.22
MOL002913	Dihydrobaicalin_qt	40.04	0.21
MOL002914	Eriodyctiol (flavanone)	41.35	0.24
MOL002915	Salvigenin	49.07	0.33
MOL002917	5,2',6'-Trihydroxy-7,8-dimethoxyflavone	45.05	0.33
MOL002927	Skullcapflavone II	69.51	0.44
MOL002928	Oroxylina	41.37	0.23
MOL002932	Panicolin	76.26	0.29
MOL002934	Neobaicalein	104.34	0.44
MOL002937	Dihydrooroxylin	66.06	0.23
MOL000612	(-)-Alpha-cedrene	55.56	0.1
MOL000073	Ent-epicatechin	48.96	0.24
MOL000131	Electron-ion collider	41.9	0.14
MOL000449	Stigmasterol	43.83	0.76
MOL000676	Dibutyl phthalate	64.54	0.13
MOL001490	Bis((2S)-2-ethylhexyl) benzene-1,2-dicarboxylate	43.59	0.35
MOL001889	Methyl linolelaidate	41.93	0.17
MOL002879	2,3-O-isopropylidene-2,3-dihydroxy-1,4-bis(diphenylphosphino)butane	43.59	0.39
MOL002897	Epiberberine	43.09	0.78
MOL003475	9-Cedranone	67.6	0.12
MOL003568	Patchoulene	49.06	0.11
MOL008206	Moslosooflavone	44.09	0.25
MOL011081	Linolenic acid methyl ester	46.15	0.17
MOL012246	5,7,4'-trihydroxy-8-methoxyflavanone	74.24	0.26

We used the STRING database to construct a PPI network to predict protein interactions. Subsequently, the significance of these genes in the network was quantified using the MCODE application in Cytoscape (fig. 2A and fig. 2B). The data indicated that SCN5A, ADRB2, CHRM3, CHRM1, CHRM2, GABRA2, and GABRA1 are the most relevant proteins (Table 2). This includes only CHRM2's neurotransmitter-related information about PD.

As shown in fig. 3A, biological process annotations suggest that *A. membranaceus* potential therapeutic mechanism in PD is primarily associated with adenylate cyclase-modulating G protein-coupled receptor signaling pathway, cellular response to acetylcholine, acetylcholine receptor signaling pathway, G protein acetylcholine coupled receptor signaling pathway. Cellular compartment annotations indicate that the action of *A. membranaceus* in PD is mainly related to compartment extrinsic component of membrane. Furthermore, molecular function annotations suggest that molecule phospholipase C activity, G-protein beta/gamma-subunit complex binding may be involved in *A. membranaceus* therapeutic effect on PD. Additionally, KEGG pathway enrichment analysis reveals that the potential therapeutic mechanism of *A. membranaceus* against PD mainly involves neuroactive ligand-receptor interaction, calcium signaling pathway, phospholipase D signaling pathway (fig. 3B and Table 3).

Based on degree centrality, target clustering analysis, and KEGG analysis, we hypothesized that CHRM2 may play a crucial role in the therapeutic effect of *Astragalus* on PD. We conducted molecular docking analysis to validate the binding of the main compounds of *Astragalus* with CHRM2. The binding energies between compounds and targets are shown in (fig. 4A and fig. 4B).

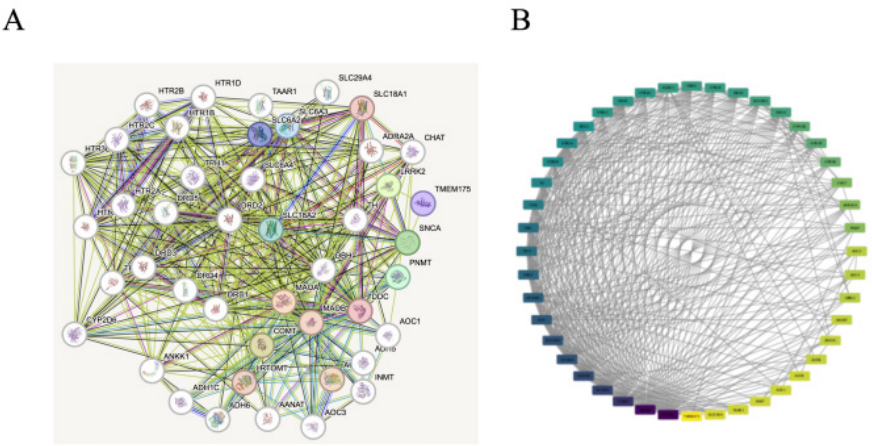

Fig. 2: Network analysis of PPI, (A): PPI network and (B): Network map of cross-target genes between active ingredients and PD-associated targets

TABLE 2: OVERVIEW OF GENES, NEUROTRANSMITTERS AND ASSOCIATED DISEASES

Gene	Description	Associated diseases	Neurotransmitter
SCN5A	Sodium voltage-gated channel alpha subunit 5A	Long QT syndrome, Brugada syndrome and cardiomyopathy	No
ADRB2	Adrenoceptor beta 2	Asthma and Chronic Obstructive Pulmonary Disease (COPD)	No
CHRM3	Muscarinic acetylcholine receptor M3	Asthma and Overactive bladder	Acetylcholine
CHRM1	Muscarinic acetylcholine receptor M1	Alzheimer's disease and PD	Acetylcholine
CHRM2	Muscarinic acetylcholine receptor M2	Alzheimer's disease and schizophrenia	Acetylcholine
GABRA2	Gamma-aminobutyric acid receptor subunit alpha-2	Alcoholism and epilepsy	GABA
GABRA1	Gamma-aminobutyric acid receptor subunit alpha-1	Epilepsy, anxiety and insomnia	GABA

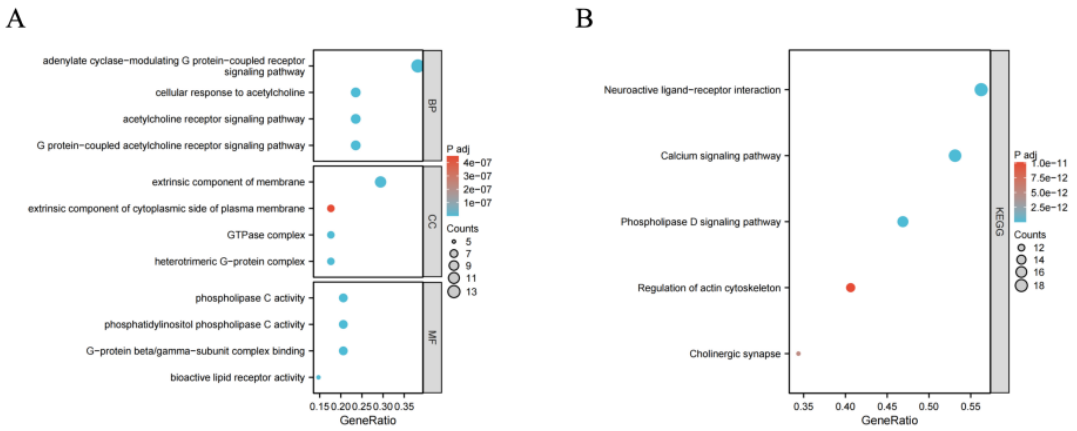

Fig. 3: GO and KEGG enrichment analysis, (A): GO and (B): KEGG pathway enrichment analysis

TABLE 3: GO ENRICHMENT ANALYSIS OF THE TOP 20 RESULTS AND KEGG PATHWAY ENRICHMENT ANALYSIS OF THE TOP 20 RESULTS

Ontology	Description	P_{adjust}	Gene ID
BP	G protein-coupled acetylcholine receptor signaling pathway	2.8236E-15	GNA15/GNAQ/CHRM2/GRK2/CHRM3/CHRM1/PLCB1/CHRM5
BP	Adenylate cyclase-modulating G protein-coupled receptor signaling pathway	5.27948E-14	GNA15/GNA12/GNAQ/CHRM2/GRK5/GNAS/CHRM3/CHRM1/LPAR3/LPAR1/GNA14/CHRM5/LPAR2
BP	Acetylcholine receptor signaling pathway	5.27948E-14	GNA15/GNAQ/CHRM2/GRK2/CHRM3/CHRM1/PLCB1/CHRM5
BP	Cellular response to acetylcholine	7.25964E-14	GNA15/GNAQ/CHRM2/GRK2/CHRM3/CHRM1/PLCB1/CHRM5
BP	Response to acetylcholine	7.73408E-14	GNA15/GNAQ/CHRM2/GRK2/CHRM3/CHRM1/PLCB1/CHRM5
CC	Heterotrimeric G-protein complex	1.68788E-09	GNA15/GNA12/GNAQ/GNAS/GNG12/GNA14
CC	GTPase complex	1.68788E-09	GNA15/GNA12/GNAQ/GNAS/GNG12/GNA14
CC	Extrinsic component of membrane	3.16186E-09	GNA15/GNA12/GNAQ/GNAS/KALRN/ARHGEF25/GNG12/GNA14/PIK3R6/PIK3R5
CC	Extrinsic component of cytoplasmic side of plasma membrane	4.49376E-07	GNA15/GNA12/GNAQ/GNAS/GNG12/GNA14
CC	Postsynaptic membrane	6.43986E-06	F2R/CHRM2/CHRM3/CHRM1/CHRM5/CHRNA5/GABRA2
MF	G-protein beta/gamma-subunit complex binding	6.08189E-13	GNA15/GNA12/GNAQ/GNAS/PLCB2/GNA14/PIK3R5
MF	Phosphatidylinositol phospholipase C activity	1.1669E-12	CHRM3/PLCB2/CHRM1/PLCB1/CHRM5/PLCB3/BDKRB2
MF	Phospholipase C activity	1.39668E-12	CHRM3/PLCB2/CHRM1/PLCB1/CHRM5/PLCB3/BDKRB2
MF	Bioactive lipid receptor activity	1.63234E-09	LPAR3/LPAR1/LPAR6/LPAR4/LPAR2
MF	Phosphoric diester hydrolase activity	4.15933E-09	CHRM3/PLCB2/CHRM1/PLCB1/CHRM5/PLCB3/BDKRB2
KEGG	Phospholipase D signaling pathway	9.24555E-17	GNA12/F2R/AGTR1/GNAS/PLCB2/PLCB1/LPAR3/LPAR1/LPAR6/LPAR5/LPAR4/LPAR2/PLCB3/PIK3R6/PIK3R5

KEGG	Calcium signaling pathway	9.24555E-17	GNA15/CYSLTR2/GNAQ/F2R/CHRM2/AGTR1/GNAS/BDKRB1/CHRM3/PLCB2/CHRM1/PLCB1/CYSLTR1/GNA14/CHRM5/PLCB3/BDKRB2
KEGG	Neuroactive ligand-receptor interaction	2.40215E-15	CYSLTR2/F2R/CHRM2/EDN2/AGTR1/BDKRB1/CHRM3/CHRM1/LPAR3/CYSLTR1/LPAR1/LPAR6/CHRM5/LPAR4/LPAR2/BDKRB2/CHRNA5/GABRA2
KEGG	Cholinergic synapse	5.12238E-12	GNAQ/CHRM2/CHRM3/PLCB2/CHRM1/PLCB1/GNG12/CHRM5/PLCB3/PIK3R6/PIK3R5
KEGG	Regulation of actin cytoskeleton	1.01446E-11	GNA12/F2R/CHRM2/BDKRB1/CHRM3/CHRM1/GNG12/LPAR1/CHRM5/LPAR5/LPAR4/LPAR2/BDKRB2

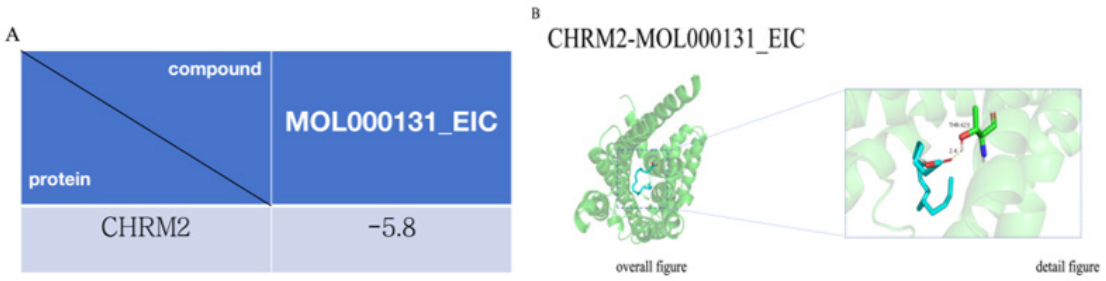

Fig. 4: Molecular docking, (A): Binding energy and (B) Interaction

A. membranaceus is believed to potentially possess neuroprotective effects against PD[20]. The active compounds within *Astragalus* are considered to exhibit anti-inflammatory and neuroprotective properties, which may contribute to alleviating neuroinflammation, safeguarding neurons from damage, and potentially playing a role in treating PD[21,22].

27 potential active compounds were identified in *Astragalus*. Using databases such as SwissTargetPrediction and Stitch, potential protein targets of active ingredients in *Astragalus* were predicted, and specific targets relevant to PD and dopamine metabolism were selected. A PPI network was constructed using the STRING database, and key proteins related to PD, including SCN5A, ADRB2, CHRM3, CHRM1, CHRM2, GABRA2, and GABRA1 were identified. Only the neurotransmitter-related information of CHRM2 was included regarding PD.

In the context of PD, CHRM2, encoding the muscarinic acetylcholine receptor M2, emerges as a significant gene of interest[23]. Acetylcholine, a neurotransmitter, is intricately involved in modulating various aspects of neuronal function, including motor control, cognition, and memory. The muscarinic acetylcholine receptors, particularly M2 subtype, play a crucial role in mediating cholinergic neurotransmission within the central nervous system. Research suggests that alterations in cholinergic signaling, including dysregulation of muscarinic receptors, could contribute to the pathophysiology of PD[24]. Specifically, CHRM2 dysfunction or alterations in its expression levels might impact cholinergic neurotransmission and subsequently influence motor and cognitive functions implicated in PD[25]. Moreover, studies have implicated muscarinic acetylcholine receptors, including M2, in the regulation of dopaminergic signaling pathways, which are central to the pathogenesis of PD[26-28]. Dysfunctional interactions between cholinergic and dopaminergic systems may exacerbate neurodegenerative processes and contribute to PD symptomatology[29,30].

The results of GO enrichment analysis indicate that *Astragalus* may exert its potential effects in the treatment of PD by modulating various biological processes, cellular components, and molecular functions. Specifically, *Astragalus* may influence biological processes relevant to PD pathology, including neurotransmitter signaling, apoptosis regulation, and inflammation modulation. In terms of cellular components, *Astragalus* effects may primarily involve the regulation of extracellular membrane structures, potentially related to cell signaling and intercellular interactions. Additionally, *Astragalus* may impact various molecular functions such as phosphatase activity, G protein-coupled receptor binding, and cytokine activity, which may

contribute to its therapeutic effects on PD.

In terms of potential therapeutic pathways, KEGG pathway enrichment analysis identified several pathways enriched in PD, including neuroactive ligand-receptor interaction, calcium signaling pathway, and phospholipase D signaling pathway. These pathways are known to be involved in dopamine neurotransmission and neuronal function regulation, suggesting that *Astragalus* may mediate its neuroprotective effects on PD by modulating the activity of these pathways.

Furthermore, the active components of *Astragalus* may also possess anti-inflammatory and antioxidant properties, which could help alleviate neuroinflammation and oxidative stress responses, thereby protecting neurons from damage[31]. Additionally, *Astragalus* may promote the expression of neurotrophic factors and synaptic formation, facilitating neuronal survival and functional recovery.

In comparison to previous studies, our research has further deepened the understanding of the mechanism of *Astragalus* in the treatment of PD. Despite being widely used in traditional medicine for the treatment of various diseases, the mechanism of *Astragalus* in PD remains relatively understudied. Through the comprehensive application of network pharmacology methods and bioinformatics analysis, our study has delved into the potential mechanisms of *Astragalus* in PD, providing a new perspective for understanding its therapeutic effects.

However, our study also has some limitations. Firstly, although we utilized advanced computational tools and databases for prediction and analysis, further experimental validation is still needed to confirm the results. Secondly, our research only explored the potential effects of *Astragalus* in a preliminary manner, and further clinical studies are required to confirm its specific therapeutic effects and dosage effects.

Therefore, while our study provides a new theoretical basis for the role of *Astragalus* in PD treatment, further research is needed to validate our findings and further elucidate its potential value in clinical practice.

This study employed a comprehensive approach integrating network pharmacology methods and bioinformatics analysis to investigate the potential mechanisms of *A. membranaceus* in treating PD. The results revealed that we specifically screened neurotransmitter-related genes associated with PD, including the CHRM2 gene. Our findings suggest that the CHRM2 gene might be one of the potential targets for *A. membranaceus* in treating PD. By modulating neurotransmitter signaling pathways, particularly the acetylcholine receptor signaling pathway, *A. membranaceus* may regulate the release and signal transduction of neurotransmitters such as dopamine, thereby exerting its neuroprotective effects.

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Conflict of interests:

The authors declared no conflict of interests.

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