European Review for Medical and Pharmacological Sciences 2022; 26: 8539-8550

Exploring the nursing effect of application Albizia bark on autism in children based on network pharmacology and molecular docking

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Abstract. – OBJECTIVE: Autism is a disorder that manifests itself in early childhood. Early diagnosis of autism may not only help the affected children themselves, but also affect family well-being and social stability. The natural drug Albizia bark has been reported to have some effect in the prevention and treatment of autism in children. Therefore, we used network pharmacology and molecular docking to explore the possible mechanism.

MATERIALS AND METHODS: TCMID and BATMAN-TCM was used to retrieve the chemical constituents of Albizia bark, and then obtained the relevant targets about autism by TTD, Gene Cards and OMIM. The resulting ingredients and targets were predicted, then a protein interaction network was constructed, and finally bioinformatics analysis was performed. Finally, molecular docking was used to verify the effective ingredients and targets obtained from the screening.

RESULTS: Leucaena saponin B, luteolin, 3', 4', 7-trihydroxyflavone, which may be the key compounds for the treatment of autism. BP mainly involving signal transduction, G protein coupled receptor signal pathway, protein phosphorylation. CC, mainly involving plasma membrane, integral component of plasma membrane, MF, including protein binding, adenosine triphosphate binding, protein kinase activity. Molecular docking showed that AKT1, HRAS, PIK3CA, PIK3R1 and SRC, five potential targets, had good binding ability to Leucaena saponin B.

CONCLUSIONS: The natural drug Albizia bark exerts pharmacological effects in a multi-component, multi-target and multi-channel manner, including neural regulation, inflammatory response and immune regulation.

Key Words:
Albizia bark, Autism, Nursing effect, Network pharmacology, Molecular docking.

Abbreviations:
PPI, Protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of genes and genomes; BP, Biological process; CC, cell composition; MF, molecular function.

Introduction

Autism is a disorder that manifests itself in early childhood with an inability to acquire social skills, repetitive behaviors, and failure in the development of verbal and nonverbal communication[1]. Pediatricians and nurses not only play an important role in the early diagnosis of autism in children, but in also influencing the development and prognosis of the disorder[2]. Early diagnosis and timely treatment of autism is not only beneficial to the child, but also affects family well-being and social stability[3]. The current prevalence of autism is reported to be about 0.76%, which means that there are about 760 children with autism for every 10,000 children[4]. In China, there are about 250 million children (0-14 years old), of which about 70 million are young children (0-3 years old). A nationwide survey of Chinese children with autism showed that the prevalence of autistic children in China is about 0.29% and is increasing[5]; according to this estimate, there are approximately 2.03 million cases of autism in early childhood in China. According to Baird et al[3], autism in children can be definitively diagnosed at the age of 2-3 years. Due to various personal, family, and social influences, some children with autism are not diagnosed in a timely manner, and many families are reluctant to admit that their child has autism[6]. This makes pediatricians and nurses often internally conflicted, reminding us of the need to spread knowledge

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about autism on the one hand, while on the other hand, the prevention and treatment of autism remains a worldwide challenge. Currently, most of the treatments related to autism are based on behavioral induction[7-9]. Chinese medicine, as a part of medicine, also plays a role in the treatment of autism[10,11]. Natural drugs can intervene and act in autism in multiple targets and have advantages for prevention and individualized treatment of autism. It is reported that the active ingredient of natural drug Albizia bark can alleviate the stress state[12]. The use of network pharmacology and molecular docking technology can provide a more powerful basis for the treatment of autism with natural drugs[13]. In this study, we used the above methods to explore and predict the effective molecular targets and potential mechanisms of Albizia bark in the treatment.

Materials and Methods

Chemical Composition Collection and Target Prediction of Albizia Bark

The chemical composition of Albizia bark was searched in the TCMID (available at: https://119.3.41.228:8000/tcmid/) and BATMAN-TCM (available at: https://bionet.ncpsb.org/batmantcm/). The literature was searched for pharmacologically active and blood-entering components for compound supplementation and screening, finally a database of bioactive components of Acacia bark was constructed. Swiss Target Prediction (available at: https://swisstargetprediction.ch/) was used for target prediction, with the species set at "Homo sapiens" in the search criteria, and targets with a probability of 0 were excluded, thus eliminating the chemical components with no relevant information.

Acquisition of Autism-Related Targets

Keywords searched were "autism", "Depression" in the TTD (available at: https://db.idrblab.net/ttd/), Gene Cards (available at: https://www.genecards.org/) and OMIM (available at: https://omim.org/). The targets associated with autism were obtained, and all targets of the three databases were integrated in Excel; duplicate genes were excluded and corrected using UniProt database.

Drug-Disease Target Prediction Results

The obtained constituent targets were mapped to each other with autism targets, and then, Veen plots were made to obtain the intersecting genes. Then Cytoscape 3.8.0 software (available at: https://cytoscape.org) was used to construct the "compound-target" network. Degree, Closeness Centrality, Betweenness Centrality were selected as the quantifiers in the network. The greater the value of Degree, Closeness Centrality and Betweenness Centrality, the more important the node is in the network. The core components were selected.

Target Protein Interaction Network Construction

To further investigate the protein interactions between Albizia bark for autism, the drug-interacting genes were uploaded to the interaction database String (available at: https://string-db.org/) for protein interaction network construction (PPI) database. The species was set at "Homo sapiens", and the minimum interaction score was set at 0.9 to ensure the credibility of this study. The other parameters remain the default settings, and the results are stored in TSV format. The TSV file was imported into Cytoscape 3.8.0, the network was analyzed, and the network analysis results were saved.

GO Enrichment Analysis and KEGG Pathway Analysis

Uploading the drug disease intersection gene into the DAVID database (Database for Annotation, Visualization and Integrated Discovery available at: https://david.ncifcrf.gov/summary.jsp – gene identifier selection: official_ GENE_ Symbol), the species setting was: Homo sapiens. Using DAVID 6.8 GO gene function we detected the role of Albizia bark in the treatment of autism and the role of target proteins in gene function thanks to three aspects: biological process (BP), cellular component (CC) and molecular function (MF). In order to clarify the target of Albizia bark in the treatment of autism, KEGG pathway enrichment analysis was carried out in the signal pathway. GO function entry and KEGG pathway entry ($p < 0.05$) were selected as the main gene function enrichment processes and signal pathways of Albizia bark in the treatment of autism, so as to predict the mechanism of Albizia bark.

Molecular Docking

Through KEGG pathway enrichment analysis, we identified potential Albizia bark related genes targeted by autism active ingredients. These tar-

Table I. Relevant parameters of core target network.

Gene	Target protein name	Degree of freedom	Near centrality	Intermediate centrality
SRC	Proto-oncogene tyrosine-protein kinase Src)	30	0.45	0.13
PIK3CA	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform	27	0.42	0.04
PIK3R1	Phosphatidylinositol 3-kinase regulatory subunit alpha	27	0.42	0.04
HRAS	GTPase HRas	26	0.45	0.09
AKT1	RAC-alpha serine/threonine-protein kinase	22	0.43	0.06
RELA	Transcription factor p65	20	0.39	0.06
JAK2	Tyrosine-protein kinase JAK2	20	0.41	0.03
HSP90AA1	Heat Shock Protein 90 Alpha Family Class A Member 1	19	0.39	0.03
IL2	interleukin 2	18	0.38	0.02
EGFR	Epidermal growth factor receptor	18	0.41	0.03
JAK1	Tyrosine-protein kinase JAK1	17	0.38	0.01
JUN	Transcription factor AP-1	17	0.39	0.03
JAK3	Tyrosine-protein kinase JAK3	16	0.38	0.09
RPS6KB1	Ribosomal protein S6 kinase beta 1	15	0.40	0.02
MAP2K1	Dual specificity mitogen-activated protein kinase 1	15	0.41	0.05
MTOR	Serine/threonine-protein kinase mTOR	14	0.39	0.02
PTK2	Focal adhesion kinase 1	14	0.37	0.09
ESR1	Estrogen receptor	13	0.37	0.01
PIK3CB	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit beta isoform	13	0.36	0.00
PTPN1	Tyrosine-protein phosphatase non-receptor type 1	13	0.36	0.04

gets were confirmed by molecular docking. Validated components were SRC, PIK3CA, PIK3R1, HRAS and AKT1. The crystal structures of the validation components were obtained from the RCSB Protein Data Bank (PDB, https://www.rcsb.org/). iGEMDOCK software was used for molecular docking. The software automatically used default parameters during standard docking. From the molecular docking results, we selected the top five receptor proteins with the lowest energy values and the ligands that most stably bound to these receptor proteins and ran Auto-Dock Vina 1.1.2 autodocking. The best scoring small molecule from each protein was selected for interaction mode analysis, and the interaction mode of the docking results was analyzed using PyMOL2.3.0 and LIGPLOT V 2.2.4.

Statistical Analysis

All differentially expressed proteins were compared to all of the experimentally identified proteins with KEGG annotation results to reveal the enriched pathways, as determined by Fisher's exact test. $p < 0.05$ was considered statistically significant.

Results

Prediction of Active Components and Targets of Albizia Bark

47 components of Albizia bark were retrieved from TCMID database and 15 from BATMAN-TCM database. After removing duplicates, a total of 47 chemical components were collected. At the same time, through literature search, for chemical components with clear pharmacological effects and blood components as candidate active components, a total of 50 bioactive components were finally screened. Swiss target prediction was used to predict, and a total of 680 targets corresponding to the composition of Albizia bark were obtained.

Autism Related Targets

91 autism related targets were obtained from TTD database, 10,367 autism related targets were obtained from Gene Cards database, and 3 autism related targets were obtained from OMIM database. Combining the data of the three databases, taking Gene Cards database as the standard, using Excel to eliminate duplicate genes, a total of 10,461 target genes were obtained, and the ob-

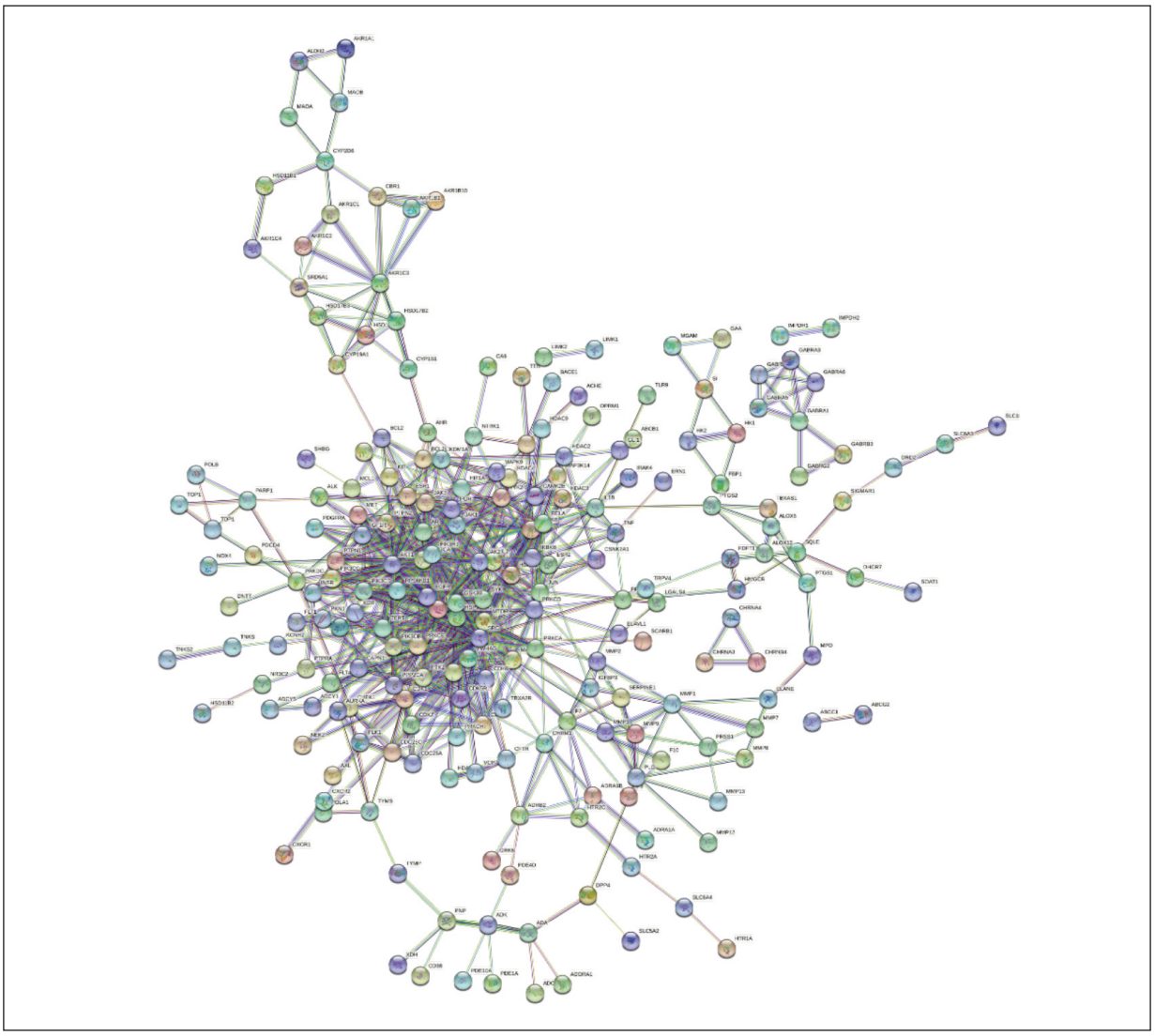

Figure 1. Key components target network diagram. Circles represent proteins, and straight lines represent interactions between protein.

tained genes were corrected by UniProt database (Supplementary Table I).

Drug Disease Target Prediction Results

Using bioinformatics & Evolutionary Genomics (available at: https://bioinformatics.psb.urgent/) the intersection of Albizia bark related compound targets and autism related targets, and a total of 294 drug disease intersection genes were obtained. Using Cytoscape 3.8.0 we built the "component target" network diagram, and screened out the key components through Cytoscape, as shown in Figure 1. The key components were: Leucaena saponin B, luteolin, 3', 4', 7-trihydroxyflavone, which may be the key compounds for the treatment of autism.

Core Target and Network Interaction

A total of 294 Albizia bark component targets obtained from Wayne diagram and autism related targets were imported into string (available at: https://string-db.org/). The protein-protein interaction was predicted in the database. The species was set as "Homo Sapiens", and the confidence was set as 0.9. Using Cytoscape 3.8.0 software, we draw the protein-protein interaction network, reflect the size and color of the target with the degree value, and reflect the thickness of the edge with the combined score value, so as to construct

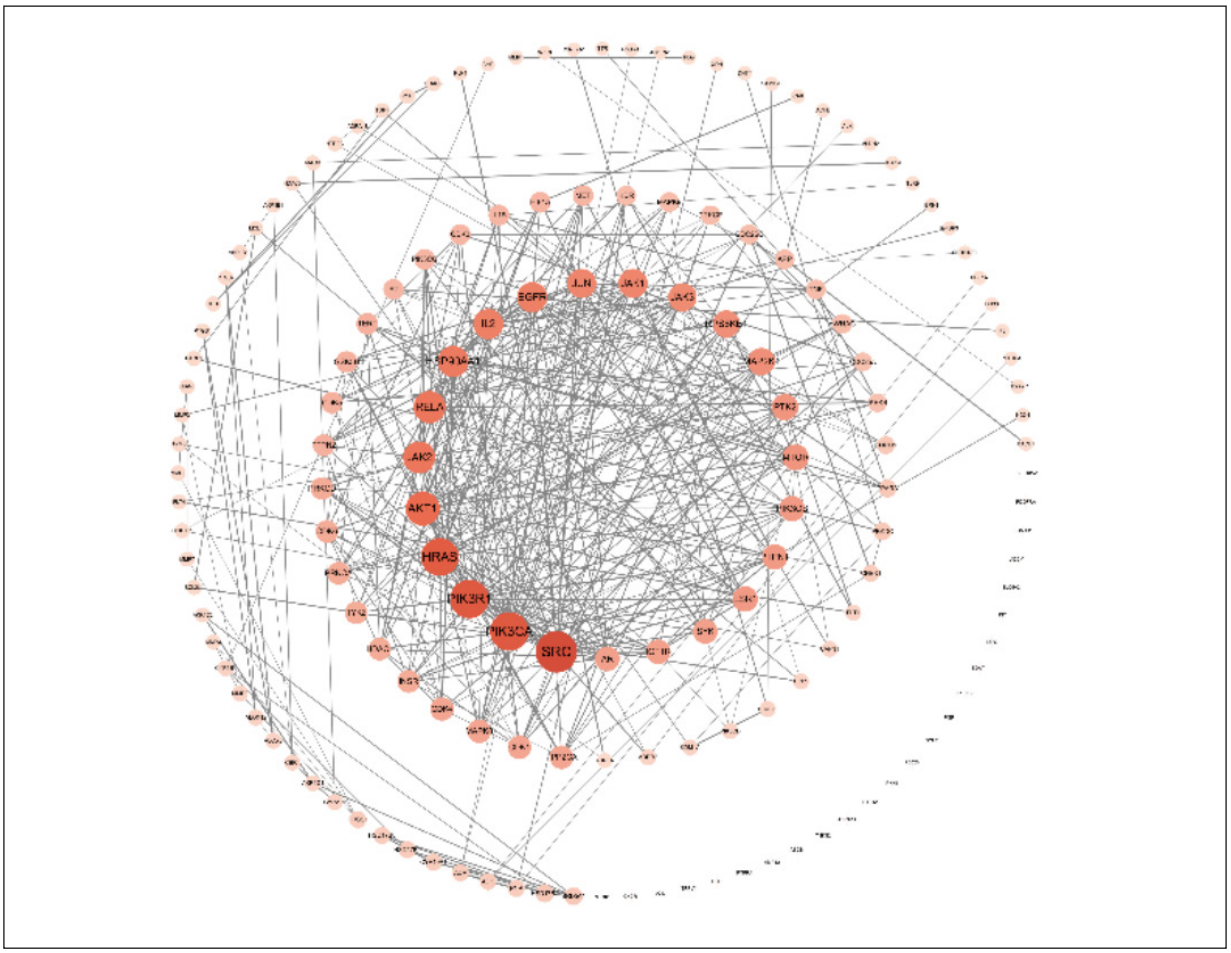

Figure 2. Protein-protein interaction diagram.

the protein-protein interaction network, as shown in Figure 2. The network had a total of 156 nodes and 418 edges. The relevant parameters of the core target network with the highest degree value are shown in Table I.

Biological Function Enrichment Analysis

Taking the drug disease intersection gene and using David database for go gene function enrichment analysis, a total of 624 go entries were screened, of which 299 were related to biological process (BP). Taking $p < 0.05$ as the standard, 30 main items with significantly enriched biological functions of Albizia bark in the treatment of autism were screened, as shown in Figure 3, mainly involving signal transduction, G protein coupled receptor signal pathway and protein phosphorylation. There are 74 cell compositions (CC), mainly involving plasma membrane, integral component of plasma membrane. Among them, 194 are related to molecular function (MF), including protein binding, adenosine triphosphate binding, protein kinase activity, etc. KEGG pathway enrichment analysis was performed in signaling pathways to elucidate Albizia bark therapeutic targets for autism. Go functional entries and KEGG pathway entries ($p < 0.05$) were selected as the main gene functional enrichment processes and signaling pathways involved in Albizia bark treatment for autism, to predict the mechanism of Albizia bark treatment for autism (Figure 4).

By using David database for pathway enrichment analysis, a total of 164 pathways related to the treatment of autism with Albizia bark were enriched. The pathways related to the treatment of autism with Albizia bark were screened according to $p < 0.05$. The pathways related to autism were screened, including neuroactive ligand receptor interaction, PI3K-Akt signal pathway, cAMP signal pathway, and other signaling pathways.

The size of the circle represents the data of genes enriched in the corresponding pathway,

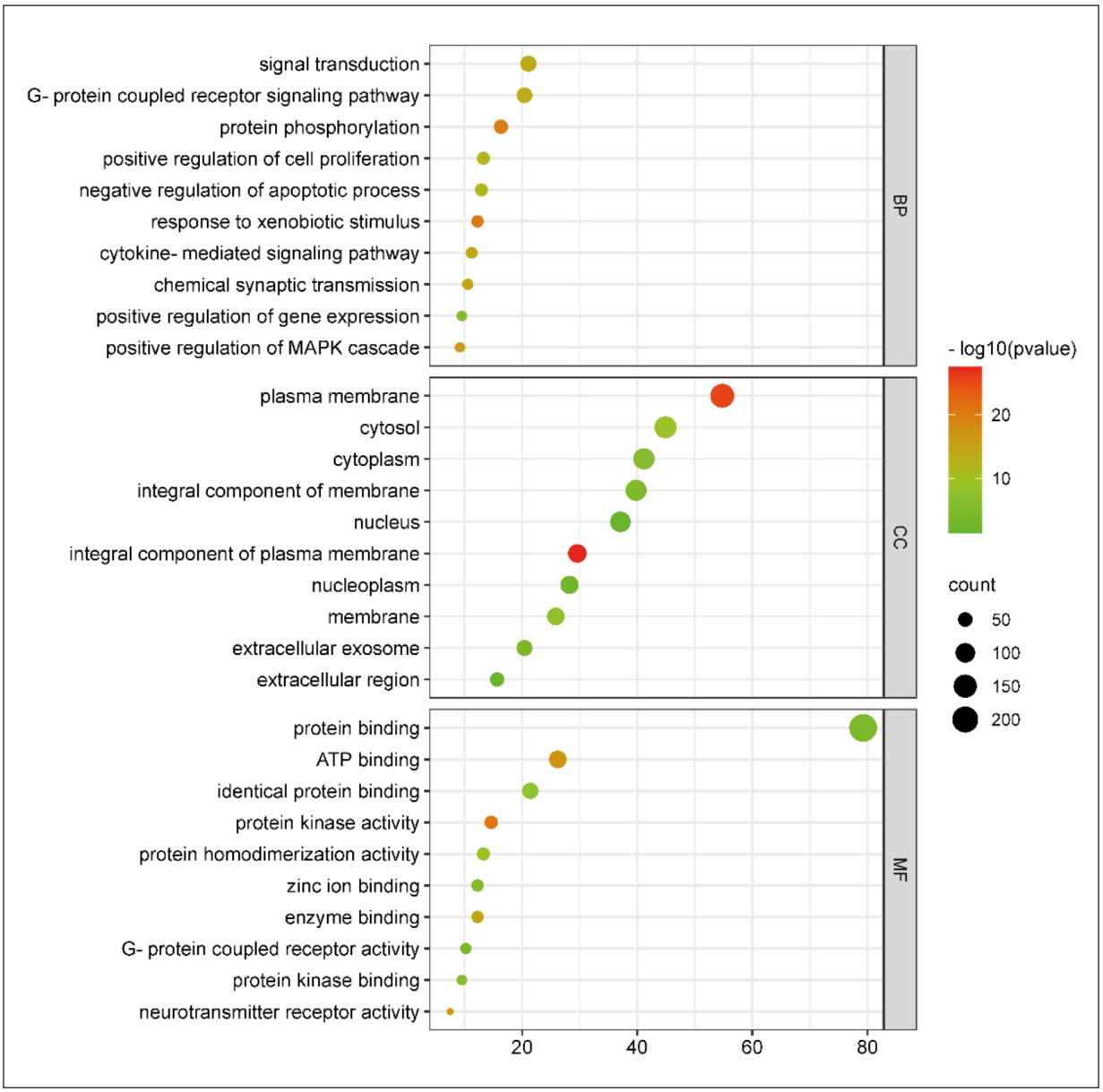

Figure 3. Go enrichment analysis of Albizia bark in the treatment of autism.

and from green to red represents that the *p*-value gradually decreases. The top 20 KEGG metabolic pathways will be screened according to the *p*-value, and the bubble diagram will be drawn according to the *p*-value. The horizontal axis is expressed by the number of genes enriched in the pathway. The size of the bubble represents the number of genes enriched in the corresponding pathway, and the depth of the color represents the significance, which can intuitively observe the significance enrichment information.

Analysis of Molecular Docking Results

According to the results of KEGG pathway enrichment analysis, we selected the neuroactive and receptor interaction signal pathway, which accounts for the largest proportion of genes involved in different biological functions and signal pathways in the total number of cross genes in autism, for further analysis. Based on the corresponding relationship between drug and target, the target protein pathway is locked by molecules. Using auto-dock Vina software, the five target

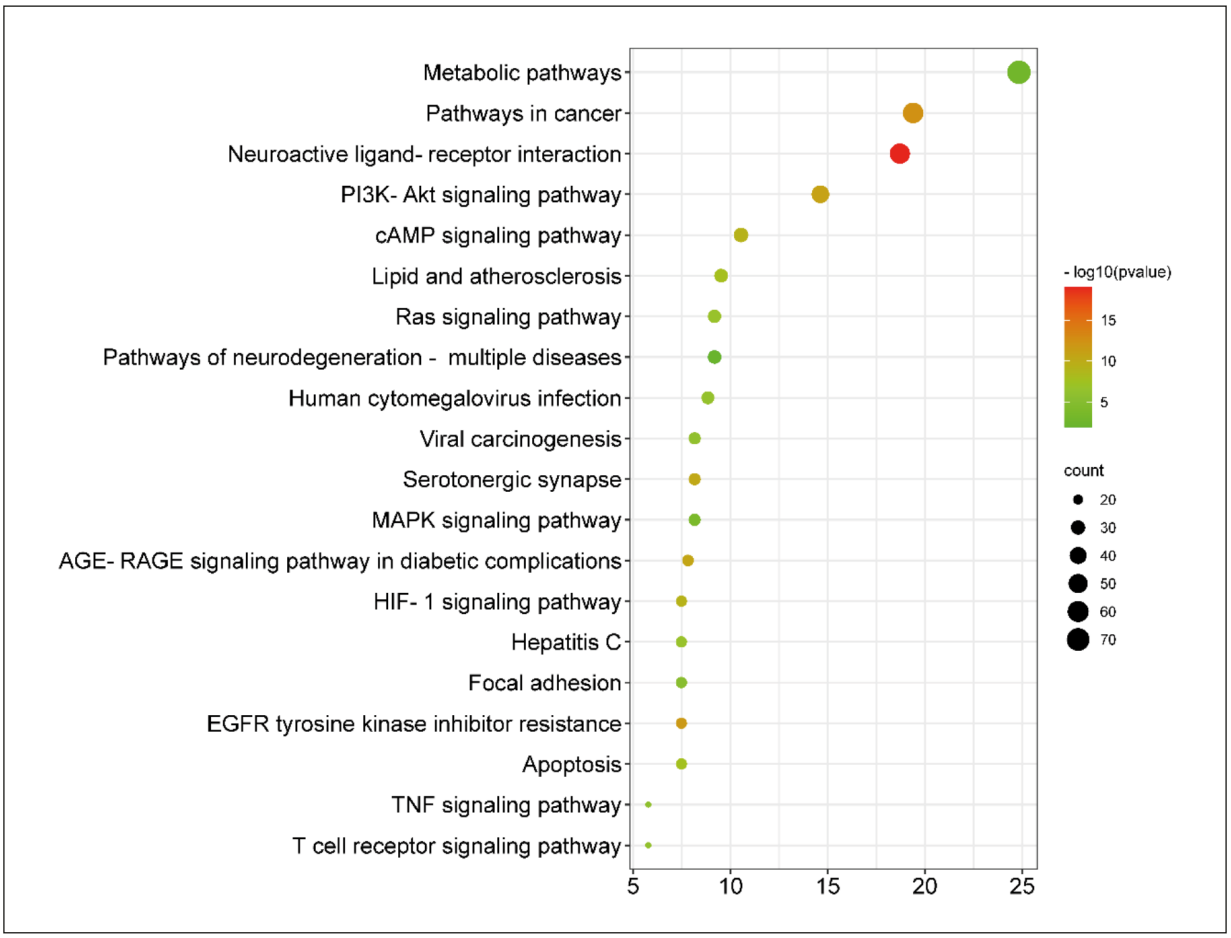

Figure 4. Bubble Diagram of KEGG enrichment pathway of Acacia bark in the treatment of autism.

proteins with the lowest energy value (AKT1, HRAS, PIK3CA, PIK3R1 and SRC) in molecular docking were connected with the active component Leucaena saponin B. Figure 5 shows the best docking combination for molecular docking. The binding energies of target protein and Leucaena saponin B, including AKT1, HRAS, PIK3CA, PIK3R1 and SRC, were -7.9, -9.1, -10.2, -9.4 and -9.4 kcal/mol, respectively. This indicates that Leucaena saponin B has good binding ability to these targets.

Discussion

The action mechanism of traditional Chinese medicine in the treatment of autism is complex, with many components and targets. When the pathogenesis has not been clarified, the method of network pharmacology allows us to systematically study the effective components, targets and pathways of drugs at the molecular level, so as to improve our understanding of the interaction between components, targets and pathways. In this study, the key components show that Leucaena saponin B, luteolin and 3', 4', 7-trihydroxyflavone may be key compounds for the treatment of autism.

This finding suggests that these components may be very important for the therapeutic effect of autism, which is worthy of further exploration. It is reported that the nervous system inflammation of autistic children is the main cause of its pathogenesis[14]. The lack of unique pathogenesis and reliable biomarkers hinders the development of effective treatment of self-diseases. Therefore, the psychopharmacological drugs prescribed to most children with autism cannot solve their core symptoms. Research[15-17] shows that the use of effective components of natural drugs can improve the symptoms of autistic children to a certain extent. Luteolin has been shown to improve

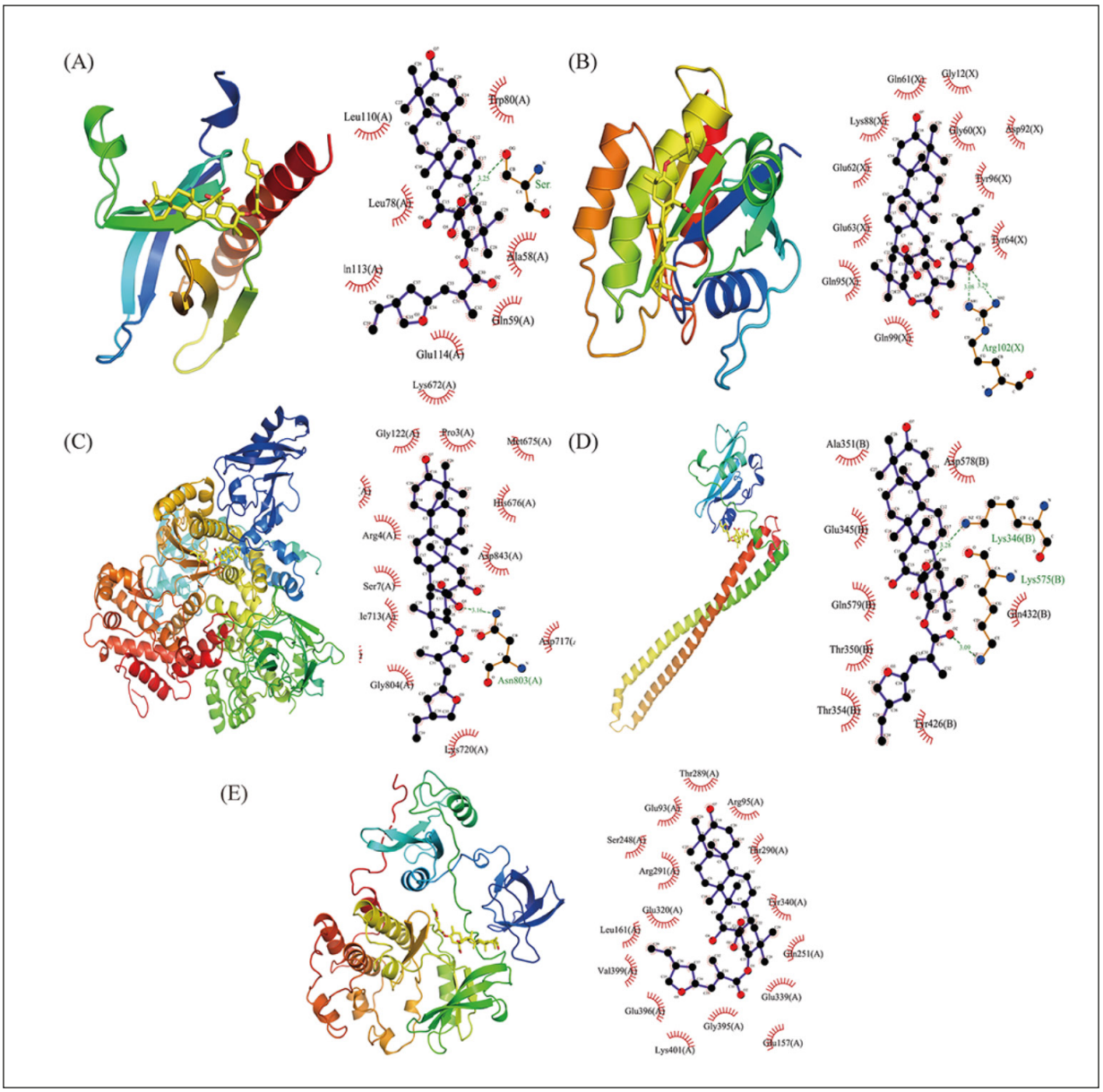

Figure 5. Molecular docking. **A**, AKT1; **B**, HRAS; **C**, PIK3CA; **D**, PIK3R1; **E**, SRC.

mental symptoms[18] and brain inflammation in children with autism[19]. Luteolin has antioxidant, anti-inflammatory, anti-allergic and neuroprotective properties, which may improve patients' oxidative stress, brain inflammation, gastrointestinal dysfunction and allergic symptoms[20,21]. Leucaena saponin B, as one of the effective components of natural drug Acacia, has been confirmed in anti-inflammatory and improving nerve injury[22]. It can not only improve pain and nerve injury in mice, but also have good performance in antidepressant[23]. It is reported that 3',4',7-Trihydroxyflavone prevents apoptotic cell death in neuronal cells from hydrogen peroxide-induced oxidative stress. Scholars[24] have shown that the neuroprotective effect of 3', 4', 7-trihydroxyflavone makes it a promising candidate for the treatment of neurodegenerative diseases. Its mechanism is mainly realized by affecting the downstream response through MAPK and PI3K/Akt signaling pathways.

In PPI network, according to the node degree, the main targets of autism are SRC, PIK3CA, PIK3R1, HRAS and AKT1. SRC plays an important role in the development and maturation of the brain[25]. The study confirmed that the normal secretion of SRC can improve the irritability of autistic children[26], and it was also found that the model mice had self-diseased behavior after SRC injury: excessive repetitive behavior and social defects[27]. Similarly, most of the reports on PIK3CA, PIK3R1 and AKT1 are accompanied by PI3K-Akt-mTOR signal pathway[28-30]. Most of these reports are related to neural mechanisms, such as brain development[28,31], brain injury[28,29,32] and childhood autism[28-31]. HRAS is reported to be associated with autism and attention deficit hyperactivity disorder[33]. Possible association of c-Harvey-Ras-1 (HRAS-1) marker with autism[34].

Biological information is one of the methods to explain the pathogenesis. It is found through bioinformatics analysis that the signal transduction[35], G protein coupled receptor signal pathway[36], protein phosphorylation[37], plasma membrane[38], integral component of plasma membrane[39], protein binding[40], adenosine triphosphate binding[41], protein kinase activity[42], all affect the occurrence and development of autism.

The signal pathway can reveal the possible mechanism of action. The three signal pathways obtained by KEGG enrichment, neuroactive ligand receptor interaction, PI3K Akt signal pathway and cAMP signal pathway, are relatively closely related to autism. Studies[28-30,43-46] showed some important pathways related to autism, which may regulate targets related to these pathways, such as neuroactive ligand receptor interaction, cAMP signaling pathway and PI3K Akt signaling pathway. This fully proves that the three signal pathways of neuroactive ligand receptor interaction, cAMP signaling pathway and PI3K Akt signaling pathway may regulate the neural development of the brain to varying degrees, inhibit nerve injury and improve the secretion and expression of some proteins, thus affecting the occurrence and development of self-diseases[47-50]. It also proves from the side that the effective components of natural drug Acacia may improve the symptoms of autistic children to a certain extent.

In order to further explore the potential molecular mechanism of Albizia bark in the treatment of autism, we used the key component Leucaena saponin B as ligand and conducted molecular docking research on five targets closely related to autism through KEGG based screening. The results showed that the five potential targets had good binding ability with Leucaena saponin B.

Of course, although this study has reached some conclusions, there are some limitations. We only discussed the role of Albizia bark in autism at the level of network pharmacology. Therefore, the results obtained in this study need to be verified in pharmacodynamics, and mechanism experiments need to be carried out to explain the complex multi-target, multi-channel and synergistic interactions involved in the treatment of autism.

Conclusions

In this study, the network pharmacology method was used to analyze the mechanism of Albizia bark in the treatment of autism. Our results show that the natural drug Albizia bark exerts pharmacological effects in a multi-component, multi-target and multi-channel manner, including neural regulation, inflammatory response and immune regulation. Our results provide a reference for the further study of the treatment mechanism of autism and a certain idea for the conservative treatment of autism with natural drugs.

Conflict of Interest

The authors declare that there are no competing interests associated with the manuscript.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Funding

No funding.

Authors' Contribution

All authors contributed to the study design and conduct. Y.-Q. Gao and L.-B. Xu, designed and wrote the manuscript; Y.-Y. Zhang, L.-L. He and X.-C. Pan analyzed the data. X.-C. Pan and L.-B. Xu drafted the manuscript and prepared the figures. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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