

Exploring the Mechanism of Bajitian in the Treatment of Autism Spectrum Disorder Based on Network Pharmacology and Molecular Docking

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Abstract: This work explored the material basis and mechanism of Bajitian for treating Autism spectrum disorder (ASD) by network pharmacology combined with molecular docking. The effective chemical components and their targets of Bajitian were collected from TCMSP. ASD-associated targets were retrieved from GeneCards. The overlapping targets of chemical components and ASD were obtained through bioinformatics platform. The network of chemical compositions-ASD targets was constructed through cytoscape software. A PPI network was established through the STRING database, and core targets were then screened using Cytoscape software. The overlapping targets were analyzed by GO and KEGG in DAVID. The molecular docking of main chemical components and core targets was performed by AutoDock software. Results showed that 19 effective chemical constituents of Bajitian and 21 targets for ASD were screened, including four major chemical components and ten core targets. Molecular docking predicted that β -sitosterol had strong binding affinity for the target proteins ACHE, SLC6A4, and ADRB2. Through GO enrichment analysis, 49 BP terms, 21 CC terms, and 22 MF terms were identified. In the KEGG analysis, eight pathways were significantly enriched, including Neuroactive ligand-receptor interaction, Cholinergic synapse, and Serotonergic synapse, etc. This study provides preliminary evidence that Bajitian may exert its therapeutic effects on ASD through multiple components, targets, and pathways. It offers a theoretical foundation for subsequent studies on the

material basis and molecular mechanisms of Bajitian as an adjunctive treatment for ASD.

Keywords: Autism Spectrum Disorder; Bajitian; Network Pharmacology; Molecular Docking

1. Introduction

Autism spectrum disorder (ASD) represents a multifaceted neurodevelopmental condition that manifests as impairments in social communication and interaction, narrow interests, and repetitive, stereotyped patterns of behavior. In addition, individuals with ASD may present with epilepsy, sleep disturbances, depression, anxiety, and gastrointestinal dysfunction, reflecting the marked heterogeneity and diversity of ASD [1]. According to the reported data, the current prevalence of ASD is 1 in 45 [2]. ASD exhibits high heritability and biological complexity, and its pathogenesis remains incompletely understood. It is generally considered to arise in genetically susceptible individuals upon exposure to specific environmental factors, and no effective treatment is currently available [3]. Currently, clinical treatment primarily relies on behavioral interventions and rehabilitation training, supplemented by both Traditional Chinese medicine and Western medicine. Western medicine mainly uses antipsychotic drugs for treatment, such as haloperidol, risperidone, and aripiprazole [4]. In Traditional Chinese medicine, therapeutic strategies typically combine pharmacological and non-pharmacological modalities. Non-pharmacological therapies include acupuncture, qigong, tuina, and auricular acupuncture, etc [5]. Traditional Chinese

medicine formulas mainly fall into three categories. The first category comprises formulas for the heart-liver fire hyperactivity type, such as Guipi Decoction and Yangxin Decoction. These formulas clear heart fire and pacify the liver. They also calm and stabilize the mind. The second category comprises formulas for the phlegm-clouding-the-heart type, including Wendan Decoction. They eliminate phlegm, resolve turbidity, open the orifices, and improve intelligence. The third category comprises formulas for kidney essence deficiency, include Liuwei Dihuang Pill, Zuogui Pill, and Yinhuo Decoction [6]. These formulas nourish yin, tonify the kidney, replenish essence, and strengthen the marrow.

Bajitian, the dried root of *Morinda officinalis*, is known as one of the four major medicinal herbs native to southern China. It is sweet, pungent and slightly warm in nature, acting on the Liver and Kidney meridians [7]. The *Shennong Bencao Jing* (Divine Farmer's Classic of Materia Medica) records that Bajitian manages severe wind-pathogen and pathogenic factors, treats impotence, strengthens tendons and bones, pacifies the five zang organs, supplements the middle jiao, and enhances willpower [8]. Bajitian is traditionally used for liver-kidney tonification, tendon and bone strengthening, and wind-dampness elimination. In clinical practice, it is mainly used to treat erectile dysfunction, nocturnal emission, cold uterus infertility, rheumatic arthralgia, and weakness and flaccidity of tendons and bones [9]. With the development of extraction technologies for traditional Chinese medicines, various active components of Bajitian have been purified and identified. Pharmacological studies suggest that Bajitian also has antidepressant, anti-Alzheimer's disease, antitumor, anti-inflammatory, and pro-angiogenic effects [10,11]. A network pharmacology study revealed that Bajitian may ameliorate the symptoms of Alzheimer's disease through multiple pathways, including neurotransmitter regulation and calcium signaling [11]. Researches have indicated that Bajitian-derived oligosaccharides alleviate depressive symptoms by regulating the PKA/pCREB/GLUT3 and BDNF/Gsk-3 β / β -catenin signaling pathways, as well as by modulating the gut microbiota [12,13]. Network pharmacology, which explores the relationships between diseases and drugs by constructing component-target-pathway networks, is a commonly used method in studies

on the pharmacological mechanisms of traditional Chinese medicines. The present work was designed to explore how Bajitian acts against autism spectrum disorder (ASD) using an integrated approach of network pharmacology and molecular docking. This research offers a theoretical basis for clarifying the pharmacodynamic material basis and molecular mechanisms by which Bajitian exerts therapeutic effects on ASD.

2. Materials and Methods

2.1 Screening of Chemical Constituents and Targets of Bajitian

The TCMSP platform was employed to screen the chemical constituents of Bajitian based on oral bioavailability (OB) $\geq 30\%$ and drug-likeness (DL) ≥ 0.18 . Putative targets of these constituents were predicted and identified from the TCMSP platform. Target names were standardized against UniProt. Human targets were then retained as the targets of the chemical constituents.

2.2 Screening of ASD-associated Targets

The GeneCards database was searched for genes using autism as the search term. The results were filtered using the median relevance score as the threshold. Duplicates were merged and removed to obtain ASD-associated targets.

2.3 Screening of Potential Targets of Bajitian for ASD Treatment

By means of the Weishengxin online platform, overlapping targets between the chemical constituents and ASD were identified. These targets served as the potential targets of Bajitian for treating ASD.

2.4 Construction of Chemical Constituents-ASD Targets Network

A chemical constituents-ASD targets network was constructed using Cytoscape 3.7.2. The topological parameters of the chemical constituents were analyzed to screen for core chemical constituents.

2.5 Construction of Protein-Protein Interaction (PPI) Regulation Networks

Potential targets of the chemical constituents for the treatment of ASD were imported into STRING 11.0. The settings were as follows, organism, *Homo sapiens*; confidence score, ≥ 0.4 ; disconnected nodes, hidden. The obtained PPI

data were loaded into Cytoscape 3.7.2. The Network analyzer tool was employed to build the PPI network, carry out topological analysis and identified core genes.

2.6 Systematic Functional Annotation Enrichment Based on GO and KEGG Databases

Using DAVID database, GO functional annotation and KEGG pathway enrichment analysis were performed. Twenty-two intersective target genes were import into the DAVID platform. The BP, MF, and CC terms were obtained with $P < 0.05$. The top ten terms with the most significant enrichment were sorted and visualized in a figure. KEGG signaling pathways were screened with $FDR < 0.05$ as the threshold, and the top ten pathways were presented graphically.

2.7 Molecular Docking

Four chemical constituents of Bajitian with a network node degree ≥ 4.0 under section 2.4 and nine core targets with a network node degree ≥ 3.7 under section 2.5 (excluding targets with unknown tertiary structures) were selected to perform molecular docking. The 3D structures of the chemical constituents were downloaded from TCMSP, and the optimal 3D protein crystal structures of the core targets were downloaded from the PDB website. Docking simulations were conducted using AutoDock, and the resulting complexes were displayed using PyMOL.

3. Results

3.1 Screening of Chemical Constituents and Target Prediction of Bajitian

As shown in Table 1, 19 chemical constituents of Bajitian were obtained, and 50 potential targets of these constituents were predicted.

3.2 Potential Targets of Bajitian for ASD Treatment

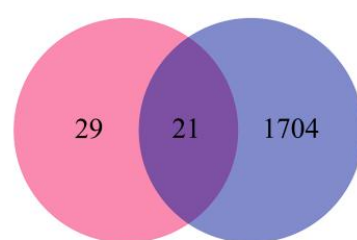
In total, 1725 ASD-related targets were retrieved from GeneCards database. Intersection analysis between chemical constituent targets and ASD-related targets produced 21 overlapping targets (Figure 1).

3.3 Chemical Constituents-ASD Targets Network

The 21 overlapping genes and the chemical constituents of Bajitian were loaded into

Cytoscape 3.7.2 to produce a chemical constituents-ASD targets network (Figure 2). The network contained 31 nodes and 43 edges. Network topological analysis revealed that the chemical constituents showed a mean degree of 4.0 in the network. Four major active chemical constituents with degree values ≥ 4.0 were identified, including beta-sitosterol (β -sitosterol, BJT11), alizarin-2-methylether (BJT14), 2-hydroxy-1, 5-dimethoxy-6-(methoxymethyl)-9, 10-anthraquinone (BJT3) and 1-hydroxy-6-(hydroxymethyl) anthraquinone (BJT4). These four constituents were used for subsequent molecular docking analysis.

Targets of chemical constituents



ASD-related targets

Figure 1. Overlapping Targets for Bajitian and ASD

Table 1. The Chemical Constituents of Bajitian

Number	Chemical constituents
BJT1	2-hydroxy-1,8-dimethoxy-7-(methoxymethyl)anthracenequinone
BJT2	1,6-dihydroxy-5-methoxy-2-(methoxymethyl)-9,10-anthraquinone
BJT3	2-hydroxy-1,5-dimethoxy-6-(methoxymethyl)-9,10-anthraquinone
BJT4	1-hydroxy-6-(hydroxymethyl)anthracenequinone
BJT5	1,5,7-trihydroxy-6-methoxy-2-(methoxymethyl)anthracenequinone
BJT6	(2R,3S)-(+)-3',5-Dihydroxy-4,7-dimethoxy-dihydroflavonol
BJT7	isoprincepin
BJT8	americanin A
BJT9	Diop
BJT10	Ohioensin-A
BJT11	beta-sitosterol
BJT12	3beta,20(R),5-alkenyl-stigmastol
BJT13	3beta-24S(R)-butyl-5-alkenyl-cholesterol
BJT14	Alizarin-2-methylether
BJT15	Ethyl oleate (NF)
BJT16	1-hydroxy-3-methoxy-9,10-anthraquinone
BJT17	2-hydroxyethyl-5-hydroxy-2-(2-hydroxybenzoyl)-4-(hydroxymethyl)benzoate
BJT18	Asperuloside tetraacetate
BJT19	Supraene

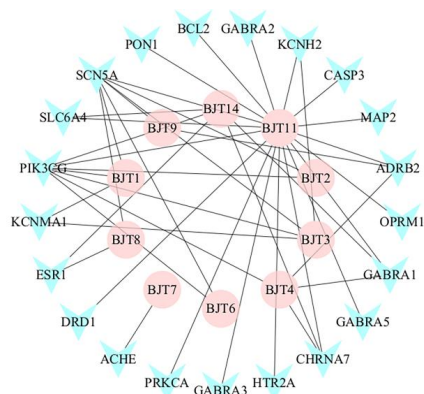


Figure 2. Chemical Constituents-ASD Targets Network

3.4 Construction of the PPI Network and Screening of Core Genes

A PPI network was built from the 21 overlapping targets using STRING (Figure 3A). It comprised 20 nodes and 37 edges. Network topological analysis showed an average node degree of 3.7. Based on a node degree ≥ 3.7 , 10 core targets were identified (Figure 3B), including acetylcholinesterase (ACHE), solute carrier family 6 member 4 (SLC6A4) and Beta-2 adrenergic receptor (ADRB2), etc.

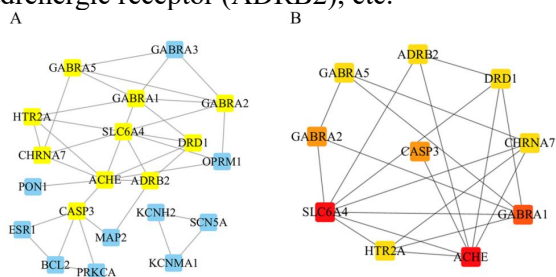


Figure 3. PPI Network Model. A: PPI Network; B: Core Targets

3.5 GO Functional Annotation and KEGG Pathway Enrichment Analysis

The DAVID database was employed for GO functional annotation of the 21 overlapping target genes. This analysis produced 92 GO terms. 49 terms were classified into the BP category, such as monoatomic ion transport and response to xenobiotic stimulus. 21 terms were assigned to the CC category, covering plasma membrane and postsynaptic membrane. 22 terms were belonged to the MF category, involving identical protein binding and GABA-A receptor activity (Figure 4).

26 signaling pathways were obtained from KEGG enrichment analysis. At an FDR threshold of < 0.05 , eight signaling pathways were identified, including neuroactive ligand-receptor interaction

and cholinergic synapse, etc. (Figure 5). These findings indicate that Bajitian may exert therapeutic effects on ASD via multiple pathways.

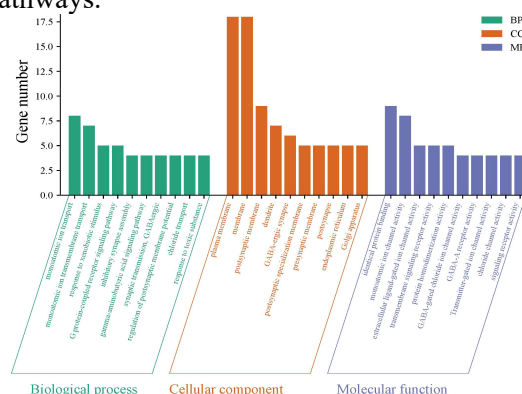


Figure 4. GO Functional Annotation

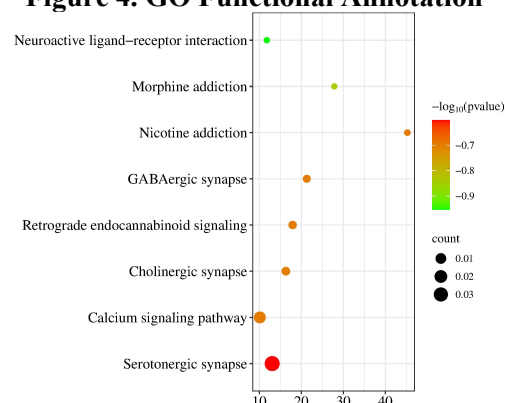


Figure 5. KEGG Pathway Enrichment Analysis

3.6 Molecular Docking

The four major active chemical constituents screened in section 3.3 and the nine core targets screened in section 3.4 were subjected to molecular docking. As shown in Figure 6, the binding energies between the major chemical constituents and the core targets varied from -10.27 to -4.41 kcal/mol, implying that the major chemical constituents of Bajitian have favorable binding affinity for the core targets and that Bajitian may act through multiple targets.

A comparison of the binding energies showed that β -sitosterol had relatively strong binding affinity for the target proteins, indicating that β -sitosterol is highly active. The binding energies of β -sitosterol with the target proteins ACHE, SLC6A4, and ADRB2 were -10.27, -9.75, and -9.17 kcal/mol, respectively (Figure 6). β -sitosterol bound to ACHE residue Tyr337 via a hydrogen bond and formed hydrophobic interactions with residues His287, Leu289, Val294. β -sitosterol bound to SLC6A4 residue Glu493 via a hydrogen bond, formed van der

Waals interactions with residues Arg104, Asn177, Ser438, and formed hydrophobic interactions with residues Tyr95, Ala169, Ile172. β -sitosterol formed van der Waals interactions with ADRB2 residues Gly90, Ile94, Thr110, and hydrophobic interactions with residues His93, Trp109, Val114 (Figure7).

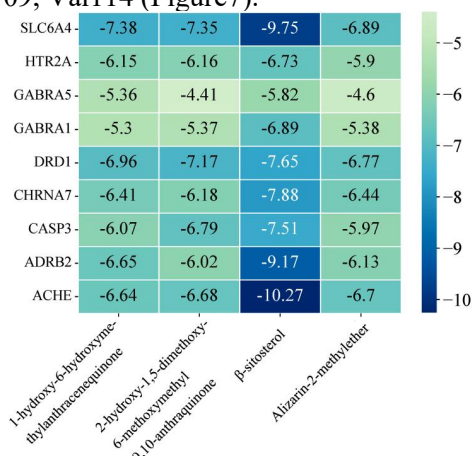


Figure 6. Binding energies of molecular docking between the constituents and targets (kcal/mol)

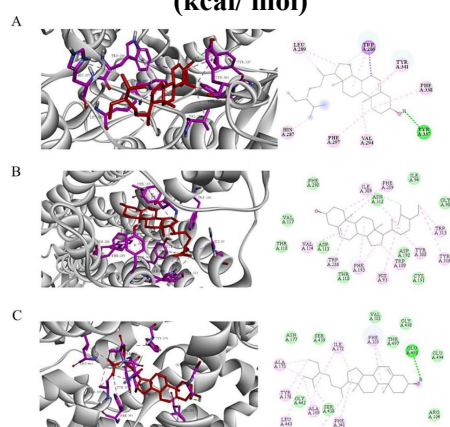


Figure 7. Molecular Docking Diagram. A: β -sitosterol and ACHE; B: β -sitosterol and ADRB2; C: β -sitosterol and SLC6A4

4. Discussion

Traditional Chinese medicines, by integrating holistic treatment with pattern differentiation and combining prevention with treatment, has achieved favorable effects in improving the behavior, language, and cognition of children with ASD. Some studies have suggested that spleen-kidney deficiency is the underlying pathogenesis of ASD and that phlegm clouding the mind is a key factor. Therefore, the therapeutic approach of “strengthening the spleen, nourishing phlegm to open the orifices and tonifying the kidney” can be adopted [14]. Clinically, modified Fugui Yizhi Decoction [14]

and augmented Yinhuo Decoction [15,16] have been used to treat ASD, with favorable therapeutic effects. As a commonly used kidney-warming Chinese medicine [17], Bajitian can also alleviate symptoms such as depression, bizarre behavior, and intellectual retardation. The present findings revealed that the active chemical constituents of Bajitian for ASD treatment included β -sitosterol, alizarin-2-methyl ether, 2-hydroxy-1, 5-dimethoxy-6-(methoxymethyl)-9, 10-anthraquinone, and 1-hydroxy-6-(hydroxymethyl) anthraquinone, etc. β -sitosterol is an active constituent widely present in medicinal plants. Owing to its unique ability to pass through the blood-brain barrier, β -sitosterol can directly affect the central nervous system and exert multiple neuroprotective effects. Studies have shown that β -sitosterol alleviates depressive symptoms by increasing the levels of brain neurotransmitters, including norepinephrine and 5-hydroxytryptamine (5-HT) [18]. In addition, β -sitosterol can specifically bind to the mitochondrial membrane, enhance the mitochondrial membrane potential and promote ATP synthesis, thereby improving neuronal energy metabolism and alleviating neurodegenerative lesions [19]. In neural stem cells, β -sitosterol promotes both proliferation and differentiation through increased expression of Hes1 and Ki-67, suggesting its potential for neural repair [20]. Alizarin-2-methoxy, 2-hydroxy-1, 5-dimethoxy-6-(methoxymethyl)-9, 10-anthraquinone and 1-hydroxy-6-hydroxymethylanthraquinone belong to the anthraquinone class of compounds. Anthraquinones act on the nervous system and possess anti-aging, antidepressant and anti-Alzheimer’s properties [21-23]. Molecular docking simulation results predicted that these four active compounds exhibit good binding affinity for the core targets, further indicating that Bajitian exerts an adjunctive therapeutic effect in the treatment of ASD through multiple active constituents and multiple targets.

Core targets including ACHE, SLC6A4, ADRB2, and others were identified through PPI network analysis. Molecular docking results indicate that β -sitosterol exhibited relatively strong binding activity with the target proteins ACHE, SLC6A4, and ADRB2. Studies have shown that ACHE possesses ion-binding activity and participates in the formation of cholinergic synapses. It affects the function of the cholinergic system by regulating acetylcholine levels in the brain. Its

abnormal activity is closely associated with the cholinergic innervation deficits detected in the fusiform gyrus of ASD patients [24]. SLC6A4 encodes the 5-hydroxytryptamine transporter (5-HTT). Polymorphisms in this gene can affect the efficiency of 5-HT reuptake. The VNTR polymorphism in its promoter region not only alters 5-HTT expression levels but may also disrupt neural circuit development, thereby increasing the risk of ASD [25]. ADRB2, an important G protein-coupled receptor, participates in cognitive and emotional regulation by modulating catecholamine signaling. Its gene polymorphisms, such as rs1042713, are associated with susceptibility to neuropsychiatric disorders and drug responses [26].

KEGG analysis indicated that the anti-ASD effect of Bajitian is associated with multiple signaling pathways. Neuroactive ligand-receptor interaction pathway comprises a group of plasma membrane receptors and ligands involved in intracellular and extracellular signaling [27]. This pathway was enriched with seven core targets of Bajitian for the treatment of ASD and may activate multiple intracellular pathways. Calcium channels are indispensable for nervous system maturation and cognitive functions, as well as learning and memory. Calcium influx can mediate the transduction of various calcium-related signals. Disruption of calcium homeostasis can lead to neuronal dysfunction, such as β -amyloid and tau protein deposition and neuronal apoptosis, thereby causing neurological disorders such as Alzheimer's disease [28]. Disruptions in the serotonergic synapse pathway can cause anxiety, depression, obsessive-compulsive disorder, phobias, and schizophrenia. Dysregulated expression of 5-HT receptors and transporters play a significant role in these conditions and have become important molecular targets for drug research. 5-HT receptors are associated with adenylyl cyclase signaling pathway, ERK1/2 signaling pathway, and mTOR signaling pathway, and participate in regulating cognition and emotion mediated by neurotransmitters such as norepinephrine, acetylcholine, and glutamate [29]. Structural changes in the active site and allosteric site of the 5-HT transporter may affect the synthesis and clearance of 5-HT and its receptors [30].

The present work employed an integrated strategy of network pharmacology and molecular docking to investigate the active constituents, targets, and signaling pathways of Bajitian in ASD treatment.

Findings from this study suggest that Bajitian may produce therapeutic benefits in ASD via a synergistic mechanism involving multiple components, targets, and pathways. These results offer a theoretical foundation and direction for future studies on the mechanisms by which Bajitian acts against ASD.

Acknowledgments

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