Herbal Medicines in Autism Spectrum Disorder: A Scoping Review of Preclinical Evidence and Therapeutic Mechanisms

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Herbal medicines present a promising therapeutic alternative for autism spectrum disorder (ASD), overcoming the limited efficacy and significant adverse effects associated with conventional drugs. This scoping review aims to summarize preclinical evidence on the use of herbal medicines in ASD treatment, focusing on types of animal models, intervention characteristics, behavioral outcomes, and mechanisms of action. A literature search was conducted in PubMed, Scopus, and Web of Science for studies published up to February 2025, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guidelines. This review included studies that were published in English and focused on herbal medicines in animal models of ASD, with evaluation of ASD-related behaviors. Quality assessment of the included studies was performed according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines 2.0. Fifty-four studies met the inclusion criteria, with 72% published from 2021 to 2025, predominantly in Asian countries (83%). Chemically induced models, particularly valproic acid-induced models (43%), were the most utilized. Single compounds (69%) were investigated more frequently than herbal extracts (22%) or traditional formulations (7%). Oral administration (48%) and therapeutic strategies (80%) were the predominant treatments. Behavioral assessments focused on social (78%) and anxiety-like behaviors (74%), with the hippocampus (43%) and cerebellum (28%) being the most frequently examined. Antioxidant (48%) and anti-inflammatory (39%) activities were the principal therapeutic mechanisms. Safety reporting was deemed to be inadequate in 81% of studies. These findings indicate that herbal medicines improve ASD symptoms primarily through modulating oxidative stress and inflammation. Future studies should address safety profiling and expanded mechanistic investigations for clinical translation.

keywords : Autism spectrum disorder, Herbal medicine, Natural compounds, Preclinical evidence, Animal models

Introduction

Autism spectrum disorder (ASD) affects approximately one in 36 children worldwide, posing considerable challenges in social communication and presenting restricted repetitive patterns of behavior, interests, or activities[1]. The complex pathophysiology of ASD involves multiple mechanisms, including oxidative stress, neuroinflammation, neurotransmitter imbalance, and synaptic dysfunction[2,3]. Currently, only two Food and Drug Administration (FDA)-approved medications (namely, risperidone and aripiprazole) are available for the treatment of specific behavioral symptoms; however, they show limited efficacy and often cause significant adverse effects, such as weight gain, metabolic disturbances, and sedation[4].

Globally, traditional medicine systems have historically employed various medicinal plants to treat neurological conditions[5]. Recent scientific investigations have demonstrated that numerous phytochemicals possess neuromodulatory properties relevant to the pathophysiology of ASD[6]. For instance, flavonoids from *Ginkgo biloba* have shown antioxidant and anti-inflammatory effects in preclinical studies[7], whereas alkaloids from *Uncaria rhynchophylla* have been demonstrated to be capable of modulating N-methyl-D-aspartate receptors[8]. Clinical evidence has suggested that herbal medicines may act as effective adjunctive therapies that enhance the therapeutic benefits of conventional pharmacological treatments for ASD[9]. However, the number of randomized clinical trials evaluating the efficacy of herbal medicines in patients with

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ASD remains limited, and existing studies have generally involved small sample sizes (fewer than 50 participants per group), suggesting that ASD remains an underexplored area within the field of herbal medicine. In recent decades, the therapeutic potential of medicinal plants for ASD has attracted increasing attention. These studies have employed various animal models, including valproic acid (VPA)-induced models, BTBR mice, and maternal immune activation models, each representing different aspects of ASD pathophysiology[10,11]. Natural compounds have shown promising effects on both the core and associated symptoms of ASD via multiple mechanisms of action, including neurotransmitter regulation, oxidative stress reduction, and neuroplasticity enhancement[12].

While several systematic reviews have previously explored the role of herbal medicines in ASD treatment[9,13], the current literature lacks a comprehensive integration of animal models, herbal interventions, molecular mechanisms, and behavioral outcomes; additionally, intervention timing strategies and sex-specific considerations in preclinical studies remain poorly characterized[14,15]. To address these limitations, we conducted a scoping review of preclinical studies examining herbal medicines and natural compounds in animal models of ASD published until February 2025. Our analysis employed a comprehensive framework that integrated the key dimensions of animal models, intervention characteristics, molecular mechanisms, and behavioral outcomes[16]. This approach provides insights into how herbal medicines affect ASD pathophysiology and identifies gaps in current preclinical research. Our work specifically examined the intersection between intervention types and their mechanisms of action across diverse animal models while critically evaluating methodological quality and safety reporting to enhance the translational potential for clinical applications.

Materials and Methods

1. Search strategies

A literature search was performed to identify studies on the effects and mechanisms of action of traditional medicines and natural products on ASD, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR)[17]. Studies were identified by searching multiple literature databases in PubMed, Scopus, and Web of Science from inception to February 2025.

The keywords and search strings used were as follows: (autism spectrum disorder OR autism) AND (herb OR herbal OR phytochemical OR plant extract OR botanical compound OR natural product OR compound OR flavonoid OR polyphenol OR alkaloid OR terpenoid OR essential oil OR nutraceutical OR phytotherapy OR medicinal plant OR ethnobotanical) AND (animal model OR animal study OR in vivo OR mouse OR rat). The search was limited to articles published in English.

2. Study selection

The inclusion criteria were as follows: studies published in scientific, academic, and peer-reviewed journals; studies on traditional medicines or natural products; studies using animal models of ASD with detailed methodological information; studies evaluating ASD-related behaviors; and studies in English. Studies were excluded if they were conference publications, clinical studies, systematic reviews, in vitro studies, or studies on outcomes without behavioral assessment. Duplicate articles were eliminated using EndNote version 20. Two independent reviewers screened the titles and abstracts of the identified studies; subsequently, a full-text review of potentially eligible articles was conducted. Any disagreements during the selection process were resolved through a consensus discussion, and a third reviewer was consulted when necessary.

3. Data extraction and analysis

Data were collected from each included study using a standardized data extraction form. The following data were extracted: publication details (authors, year, and country), animal model characteristics (species, strain, sex, and induction method), intervention details (compound/extract type, dose, route of administration, treatment timing, and duration), behavioral assessments (test types and outcomes), brain regions examined, molecular mechanisms investigated, and safety reports. The extracted data were categorized and analyzed to identify patterns and trends across studies. Descriptive statistics (frequencies and percentages) were calculated to characterize the distribution of the study characteristics and findings.

4. Quality assessment of included studies

The quality of the selected studies was evaluated using the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines 2.0 for reporting animal research[16]. The ARRIVE guidelines consist of 20 items: (1) study design, (2) sample size, (3) inclusion and exclusion criteria, (4)

randomization, (5) blinding, (6), outcome measures, (7) statistical methods, (8) experimental animals, (9) experimental procedures, (10) results, (11) abstract, (12) background, (13) objectives, (14) ethical statement, (15) housing and husbandry, (16) animal care and monitoring, (17) interpretation/scientific implications, (18) generalizability/translation, (19) protocol registration, and (20) data access. Each study was rated as "2", "1", and "0" equivalent to "clearly sufficient," "possibly sufficient," and "clearly insufficient" for each item. Two independent reviewers performed the quality assessment to minimize subjective bias, and any discrepancies in scoring were resolved through consensus discussions with a third reviewer when necessary.

Results

1. Literature search outcomes

The study selection process is illustrated in Fig. 1. A total of 2,780 records were identified in PubMed, Web of Science, and Scopus databases. After eliminating duplicate studies, titles and abstracts of 2,162 articles were screened, among which 125 articles were selected for a detailed full-text review. Finally, 54 original articles met the inclusion criteria and were included in the final analysis.

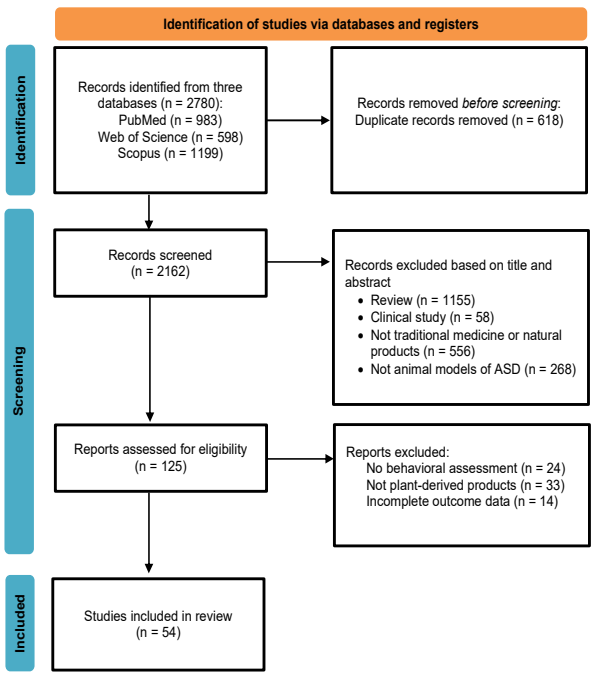


Fig. 1. Flow diagram of literature search and study selection process.

2. Temporal and geographical distribution of included studies

Preclinical studies investigating herbal medicines for ASD exhibited a clear upward trajectory over the study period (Fig. 2A). Out of the 54 included studies, 39 (72.22%) were published within the most recent 5-year period (2021–2025). Year-by-year analysis revealed minimal research output (≤1 study annually) until 2014, followed by a gradual increase beginning in 2017. Research significantly accelerated from 2023 to 2024, with 11 studies (20.37%) published in 2023 and 16 studies (29.63%) published in 2024, collectively accounting for 50.00% of all included studies. Such recent acceleration likely reflects the growing scientific interest in alternative therapeutic approaches for ASD management.

The geographical distribution of studies revealed a significant regional imbalance (Fig. 2B). Research from Asian countries was predominant (45 studies, 83.33%), followed by North America (three studies, 5.56%), Europe (two studies, 3.70%), and South America (two studies, 3.70%). Country-specific analysis identified India (14 studies, 25.93%), China (11 studies, 20.37%), and Iran (9 studies, 16.67%) as the leading contributors, collectively accounting for 62.97% of all studies. This distribution pattern may reflect the strong historical foundation of traditional herbal medicines in these regions.

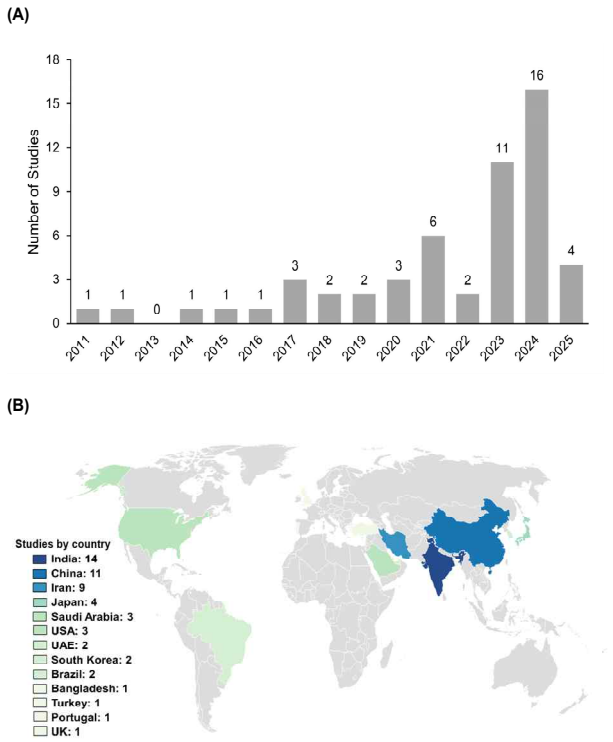


Fig. 2. Temporal (A) and geographical (B) distribution of included studies. All studies published up to February 2025 were included in the analysis.

3. Experimental procedures and outcomes

Tables 1–3 provide details of the experimental procedures and outcomes of the 54 studies included in this review, including animal models, intervention characteristics, and behavioral and biological assessments.

1) Animal models

The animal models used across the 54 studies were categorized into four types: genetic, chemically induced, stress-induced, and surgical (Fig. 3). Analysis of animal species revealed that mice were used more frequently (31 studies, 57.41%) than rats (23 studies, 42.59%). Chemically induced models were predominant (34 studies, 62.96%), with VPA models being the most frequent (23 studies, 42.59%). These included prenatal (18 studies, 33.33%) and postnatal (five studies, 9.26%) VPA injections. Propionic acid models were the next most common (eight studies, 14.81%), whereas other chemically induced models appeared in single studies. Genetic models appeared in 11 studies (20.37%), primarily BTBR mice (8 studies, 14.81%), followed by Scn1a+/− mutant mice (2 studies, 3.70%) and CDKL5R59X knock-in mice (1 study, 1.85%). Study 27 employed both BTBR mice and postnatal VPA models, and thus appeared in multiple categories. Stress-induced models were used in 8 studies (14.81%), including maternal immune activation (3 studies, 5.56%) and maternal separation stress (5 studies, 9.26%). One study (1.85%) used a surgical model of ovariectomized mice.

Sex-specific analysis revealed a substantial imbalance, with 41 studies (75.93%) exclusively using male animals, 12 studies (22.22%) including both sexes, and only one study (1.85%) focusing solely on females (Fig. 3). This marked sex disparity suggests potential limitations in understanding sex-specific responses to herbal interventions in ASD models.

Table 1. Characteristics of included studies using single compounds.

Compound	Source	Study	Animal model	Sex	Age	Number of groups	Animals per group	Positive control	Route of administration	Treatment timing	Dose	Treatment duration	Main findings	Molecular/biological markers
Polyphenols (flavonoids, phenolic acids, stilbenes, and other polyphenols)														
Alpha-glycosyl isoquercitrin	citrus fruits, red beans, buckwheat	Okano, 2022[18]	postnatal LPS-injected rat	male	3 days	4	6–10	none	mix with diet	preventive and therapeutic	0.25%, 0.5%	77 days	mother-child communication ↑, social interaction ↑	Iba1 ↓, CD68 ↓ GFAP ↓, MDA ↓, NeuN ↑, p-ERK1/2 ↑
Apigenin	parsley, chamomile, celery	Jayaprakash, 2024[19]	BTBR mice	male	8 weeks	10	5–6	none	intraperitoneal	therapeutic	10, 20, 30 mg/kg	21 days	repetitive behavior ↓, self-grooming ↓, hyperactivity ↓	choline-induced current ↑, choline-induced GABA amplitudes and frequencies ↑, SOD ↑, CAT ↑
Catechin	green tea	Mehta, 2021[20]	propionic acid-injected rat	male	1 day	11	6–8	none	oral	therapeutic	25, 50, 100 mg/kg	27 days	repetitive and stereotyped behaviors ↓, hyperlocomotor activity ↓, social interaction ↑, anxiety ↓, depression ↓, learning and memory ↑	LPO ↓, nitrile ↓, GSH ↑, CAT ↑, SOD ↑, mitochondrial complex (I, II, IV) ↑, IL-1β ↓, TNF-α ↓, IFN-γ ↓, NF-κB ↓, caspase 3 ↓, homocystein ↓, HSP-70 ↓, iNOS ↓
Curcumin	Curcuma longa	Jayaprakash, 2021[21]	BTBR mice	male	11–13 weeks	8	7	none	intraperitoneal	therapeutic	25, 50, 100 mg/kg	21 days	sociability ↑	choline-induced current ↑, choline-induced GABA amplitudes and frequencies ↑, SOD ↑, CAT ↑
Fisetin	various fruits, vegetables, trees, and shrubs	Mehra, 2023[22]	prenatal VPA-injected rat	male	3 days	10	9	none	oral	preventive and therapeutic	10 mg/kg	10 days	sensorimotor ↑, repetitive and stereotyped behaviors ↓, social interaction ↑	ROS ↓, LPO ↓, CAT ↑, SOD ↑, GSH ↑, activity of ATPases, AChE ↑,
Fisetin	various fruits, vegetables, trees, and shrubs	Mehra, 2024[23]	prenatal VPA-injected rat	male	24 days	6	9	none	oral	preventive	10 mg/kg	10 days	social behavior ↑, anxiety ↓	mitochondrial complex (I-IV) ↑, BBB permeability ↓, apoptosis ↓, WNT3A ↓, FZD6 ↓, CTNNB1 ↓, GSK3B ↑, CLDN3 ↑, CLDN5 ↑, CLDN2 ↓, CASP3 ↓

Genistein	dyer's broom, soy	Kumar, 2025[24]	propionic acid-injected rat	male and female	6 months	8	8	aripiprazole 5mg/kg	oral	therapeutic	40, 80 mg/kg	33 days	locomotion ↑, neuromuscular coordination ↑, depression ↓, memory and cognition ↑	AC ↑, cAMP ↑, CREB ↑, PKA ↑, mitochondrial complex (I-V) ↑, IL-1β ↓, TNF-α ↓, Bcl-2 ↑, Bax ↓, caspase 3 ↓, MBP ↓, NEFL ↓, glutamate ↓, serotonin ↑, GABA ↑, dopamine ↑, acetylcholine ↑, brain injury ↓
Hesperetin and nano-hesperetin	citrus fruits	Khalaj, 2018[25]	prenatal VPA-injected rat	male	28 days	6	7	none	oral	preventive and therapeutic	10 and 20 mg/kg	51 days	anxiety ↓, social interaction ↑	MDA ↓, GSH ↑, activity of SOD, CAT, GPx, GRx ↑, IL-6 ↓, TNF-α ↓
Proanthocyanidin	grape seed	Alhusain, 2024[26]	BTBR mice	male	5–7 weeks	4	8	none	oral	therapeutic	100 mg/kg	4 weeks	social interaction ↑, repetitive and stereotyped behaviors ↓, anxiety ↓	DNA strand break ↓, ROS ↓, GSH/GSSG ↑, Gadd45a ↓, Parp1 ↓, Xrcc1 ↑, Ogg1 ↑
Puerarin	kudzu root	Jiang, 2024[27]	postnatal VPA-injected mice	male	12 days	6	n/a	none	intraperitoneal	preventive and therapeutic	75, 100, 125 mg/kg	4 days	social interaction ↑, repetitive and stereotyped behaviors ↓, memory and cognition ↑	DCX ↑, SOX2 ↑, Ddx5 ↑, Ptgs2 ↑, Neu4 ↑, Sat1 ↓, Hmox1 ↓, FoxO3 ↓, MDA ↓, Fe2+ ↓,4-Hne ↓, Acsl4 ↓ GSH ↑, Nrf2 ↑, Slc7a11 ↑, GPX ↑, FTH1 ↑
Quercetin	apples, red onions, grapes, citrus fruits, cherries, broccoli, and capers	Fan, 2024[28]	maternal immune activation in mice	male	8 weeks	5	6	none	oral	therapeutic	30 mg/kg	4 weeks	stereotyped behaviors ↓, anxiety ↓, social interaction ↑, cognition and memory ↑	MMP9 ↓, TRAF6 ↓, IL-17A ↓
Quercetin	apples, red onions, grapes, citrus fruits, cherries, broccoli, and capers	de Mattos, 2020[29]	prenatal VPA-injected rat	male and female	30 days	4	10	none	intragastric	preventive	50 mg/kg	13 days	social interaction ↑, nociception ↑	ROS ↓, nitrile ↓, TBARS ↓, CAT ↑, SOD ↑, activity of GST and ALA-D ↑
Quercetin nanophytosomes	apples, red onions, grapes, citrus fruits, cherries, broccoli, and capers	Moghaddam, 2023[30]	maternal separation stress in rat	male	9 days	7	6	none	oral	therapeutic	10 and 40 mg/kg	21 days	repetitive and stereotyped behaviors ↓, anxiety ↓, social interaction ↑	MDA ↓, CAT ↑, SOD ↑, GPx ↑, GSH ↑, Nrf2 ↑, Bcl-2 ↑, Bax ↓, caspase 3 ↓
Resveratrol	grapes, berries, peanuts, and red wine	Bakheet, 2017[31]	BTBR mice	male	6–8 weeks	6	6	none	intraperitoneal	therapeutic	20 and 40 mg/kg	7 days	repetitive behavior ↓	Foxp3 ↑, T-bet ↓, GATA-3 ↓, RORγt ↓, IL-17A ↓
Resveratrol	grapes, berries, peanuts, and red wine	Xie, 2018[32]	prenatal progestin-exposed rat	male and female	5 weeks	4	5	none	oral	preventive and therapeutic	20 mg/kg	28 days	repetitive behavior ↓, social interaction ↑	ERβ ↑, SOD2 ↑, ERRα ↑, O2− ↓, 3-NT ↓, 8-OHdG ↓, γH2AX ↓, palmitate oxidation ↓, H3K9me2 ↓, H3K27me3 ↓
Resveratrol	grapes, berries, peanuts, and red wine	Zeng, 2024[33]	maternal immune activation in mice	male	8–12 weeks	3	6	none	intraperitoneal	preventive	20 mg/kg	12 h prior to maternal immune activation	repetitive and stereotyped behaviors ↓, anxiety ↓, social interaction ↑	THOC5 ↑, IL-6 ↓, Iba-1 ↓
Resveratrol	grapes, berries, peanuts, and red wine	Bhandari, 2017[34]	propionic acid-injected rat	male	3–4 months	5	5	none	oral	therapeutic	5, 10, 15 mg/kg	4 weeks	repetitive and stereotyped behaviors ↓, anxiety ↓, depression ↓, social	LPO ↓, CAT ↑, SOD ↑, GSH ↑, activity of mitochondrial enzyme complex I, II, IV ↑, TNF-α

													interaction ↑, spatial learning and memory ↑	↓, MMP-9 ↓
Resveratrol	grapes, berries, peanuts, and red wine	Shahrbabaki, 2023[35]	prenatal VPA-injected rat	male and female	30 days	6	14	none	intraperitoneal	preventive	3.6 mg/kg	12 days	anxiety ↓, social interaction ↑	n/a
Rosmarinic acid	Boraginaceae and Lamiaceae plant species	Mahmoudian, 2024[36]	maternal separation stress in mice	male	2 days	5	6–8	none	intraperitoneal	therapeutic	1, 2, 4 mg/kg	14 days	passive avoidance memory ↑, social interaction ↑, repetitive and compulsive-like behavior ↓	nitrile ↓, IL-1β ↓, TNF-α ↓, TLR4 ↓, iNOS ↓
Syringic acid	honey, olives, grapes, radish	Mallan, 2023[37]	prenatal VPA-injected rat	male	25 days	5	6–8	none	oral	therapeutic	25, 50, 100 mg/kg	4 weeks	motor coordination ↑, pain sensitivity ↑, hyperactivity ↓, social interaction ↑	IL-6 ↓, TNF-α ↓, LPO ↓, GSH ↑, CAT ↑, glutamate ↓, GABA ↑, p38 MAPK ↓, neuronal apoptosis ↓
Vanillic acid	Angelica sinensis root	Farzan, 2023[38]	maternal separation stress in rat	male	2 days	5	8	none	intraperitoneal	therapeutic	25, 50, 100 mg/kg	14 days	anxiety ↓, depression ↓, repetitive and compulsive behaviors ↓, memory ↑	antioxidant capacity ↑, MDA ↓, TLR4 ↓, NLRP3 ↓, IL-1β ↓, TNF-α ↓, CA3 diameter ↑, percentage of dark neurons ↓
Terpenoids (monoterpenoids, diterpenoids, triterpenoids, carotenoids)														
Astaxanthin	microalgae	Al-Amin, 2015[39]	prenatal VPA-injected mice	male and female	25 days	4	10	none	oral	therapeutic	2 mg/kg	4 weeks	pain sensitivity ↑, hyperlocomotor activity ↓, social interaction ↑	MDA ↓, NO ↓, GSH ↑, CAT ↑, SOD ↑, APOP ↓
Astragaloside IV	Astragali Radix	Chen, 2025[40]	BTBR mice	male	3 months	4	10	none	intraperitoneal	therapeutic	20–50 mg/kg	7 days	repetitive behavior ↓, social interaction ↑	vGLUT1/glutamate ↓, vGAT/GABA ↑, p-CREB ↑, p-CaMKIIα ↑
Crocin	Crocus sativus (saffron)	Seyedinia, 2023[41]	prenatal VPA-injected rat	male	30 days	6	6	none	intraperitoneal	therapeutic	15, 30 mg/kg	4 weeks	spatial memory ↑, pain sensitivity ↑, balance ability ↑, anxiety ↓	MDA ↓, GSH ↑, CAT ↑
Fucoxanthin	seaweed	Anand, 2024[42]	prenatal VPA-injected rat	male	3 weeks	6	6	none	oral	therapeutic	50, 100, 200 mg/kg	3 weeks	spatial memory and learning ↑, pain sensitivity ↑, hyperactivity ↓, balance ability ↑, motor coordination ↑, anxiety ↓, social interaction ↑	IL-1β ↓, TNF-α ↓, IL-17 ↓, GABA ↑, glutamate ↓, GSK-3β ↑, Akt ↓
Lycopene	tomatoes	Erten, 2021[43]	propionic acid-injected rat	male	3 weeks	5	7	none	oral	therapeutic	5, 10, 20 mg/kg	35 days	learning and memory ↑	MDA ↓, IL-1α ↓, IL-8 ↓, TNF-α ↓, NF-kB ↓, HO-1 ↑, Nrf2 ↑
Tanshinone-IIA	Salvia miltiorrhiza	Sherawat, 2024[44]	propionic acid-injected rat	male and female	6-8 months	8	8	none	intraperitoneal	therapeutic	30, 60 mg/kg	44 days	locomotion performance ↑, depression ↓, neuromuscular coordination ↑, memory and cognition ↑	c-JNK ↓ , p38MAPK ↓, IL-1β ↓, IL-6 ↓, TNF-α ↓, BDNF ↑, Bcl-2 ↑, glutamate ↓, serotonin ↑, GABA ↑, dopamine ↑, acetylcholine ↑, injury volume ↓
Thymol	Thyme	Xiong, 2023[45]	prenatal VPA-injected rat	male	22 days	6	10	none	oral	therapeutic	15, 30, 60 mg/kg	7 days	motor activity ↑, social interaction ↑	IL-1β ↓, TNF-α ↓, PSD95 ↑, SYP ↑, Pin1 ↓, p-p38MAPK ↓

Cannabinoids														
Cannabidiol	cannabis	Kaplan, 2017[46]	Scn1a+/− mutant mice	male and female	21 days	8	8–14	none	intraperitoneal	therapeutic	10–200 mg/kg	7 days (twice daily) or 1 h before behavioral tests	seizure ↓, social interaction ↑	GABAergic neurotransmission ↑, excitatory output ↓
Cannabidiol	cannabis	Pedrazzi, 2025[47]	prenatal VPA-injected mice	male	8 weeks	6	7–8	none	intraperitoneal	therapeutic	30, 60 mg/kg	30 min before behavioral tests	repetitive behavior ↓, social interaction ↑, learning and memory ↑	n/a
Cannabidiol	cannabis	Li, 2024[48]	CDKL 5R59X knock-in mice	male	12–16 weeks	4	6–14	none	intraperitoneal	therapeutic	30, 60 mg/kg	1 h before behavioral tests	social interaction ↑, memory ↑	CB1R ↓ and TRPV1 ↓ in hippocampus; TRPV1 ↑ and TRPV2 ↓ in cortex
Cannabidiol	cannabis	Patra, 2020[49]	Scn1a+/− mutant mice	male and female	8 days	3	10–11	none	subcutaneous	therapeutic	100 mg/kg	44 days	social interaction ↑, anxiety ↓, depression ↓, cognition ↑	n/a
Cannabidiol and cannabis-inspired terpene blends	cannabis	Staben, 2023[50]	BTBR mice	male and female	80 days	11	8	none	inhalation	therapeutic	0.51 mg per 6 s vapor pull	6 s vapor pull, every 5 min for 30 min	anxiety ↓, social interaction ↑	n/a
Alkaloids														
Lotusine	Nelumbinis Plumula	Liu, 2024[51]	propionic acid-injected mice	male	6–8 weeks	5	8	none	oral	therapeutic	5, 10, 20 mg/kg	5 days	social interaction ↑, learning and memory ↑	c-fos ↑, p-GluA1 Ser 845 ↑, p-GluA1 Ser 831 ↑,
Papaverine	opium poppy	Luhach, 2021a[52]	prenatal and postnatal 5-methoxytryptamine-injected rat	male	0 day	5	8	none	intraperitoneal	therapeutic	15, 30 mg/kg	4 weeks	hyperlocomotor activity ↓, social interaction ↑, repetitive behavior ↓, anxiety ↓	BDNF ↑, p-CREB ↑, IL-6 ↓, TNF-α ↓, IL-10 ↑, TBARS ↓, GSH ↑
Papaverine	opium poppy	Luhach, 2021b[53]	prenatal VPA-injected rat	male	21 days	6	8	none	intraperitoneal	therapeutic	3, 10, 30 mg/kg	4 weeks	hyperlocomotor activity ↓, social interaction ↑, repetitive behavior ↓, anxiety ↓, pain sensitivity ↑	synapsin-IIa ↑, DCX ↑, BDNF ↑, pCREB/CREB ↑, IL10 ↑, GSH ↑, IL-6 ↓, TNF-α ↓, TBARS ↓
Piperine	black pepper (Piper nigrum) and long pepper (Piper longum)	Pragnya, 2014[54]	postnatal VPA-injected mice	male and female	14 days	5	6	none	subcutaneous	therapeutic	5, 20 mg/kg	4 weeks	sensorimotor ↑, thermal nociception ↑, locomotor activity ↓, motor coordination ↑, social interaction ↑, anxiety ↓, learning and memory ↑	serotonin ↑, MDA ↓, nitrile ↓, GSH ↑, damage to Purkinje cell layer ↓
Others														
Glucoraphanin	cruciferous vegetable	Fujita, 2020[55]	maternal immune activation in mice	male	4 weeks	4	8–9	none	mix with diet	preventive and therapeutic	0.10%	4 weeks	social interaction ↑, cognition ↑	parvalbumin ↑
Ligustilide	Angelica Sinensis and Chuanxiong	Zhou, 2024[56]	postnatal VPA-injected mice and BTBR mice	male	14 days	6	10	risperidone 2.5 mg/kg	intraperitoneal	preventive (VPA model) and therapeutic (BTBR model)	10 and 30 mg/kg	3 days (VPA model) and 7 days (BTBR model)	repetitive and stereotyped behavior ↓, social interaction ↑, motor function ↑	ferritinophagy markers (ATG5, ULK1, LC3B-II, Beclin1, NCOA4) ↓
Umbelliprenin	Ferula species	Karimi, 2023[57]	maternal separation stress in mice	male	2 days	4	15	none	intraperitoneal	therapeutic	12.5, 25 mg/kg	7 days	passive avoidance memory ↑, social interaction ↑,	antioxidant capacity ↑, MDA ↓, nitrile ↓, MECP2 ↑

												repetitive behavior ↓, anxiety ↓	

Note: LPS: lipopolysaccharide; VPA: valproic acid; GFAP: glial fibrillary acidic protein; Iba1: ionized calcium-binding adapter molecule 1; MDA: malondialdehyde; NeuN: neuronal nuclei; ERK: extracellular signal-regulated kinase; IL: interleukin; TNF: tumor necrosis factor; GABA: gamma-aminobutyric acid; SOD: superoxide dismutase; CAT: catalase; NO: nitric oxide; GSH: glutathione; APOP: apoptogenic protein; vGLUT1: vesicular glutamate transporter 1; vGAT: vesicular GABA transporter; cAMP: cyclic adenosine monophosphate; CREB: cAMP response element-binding protein; CB1R: cannabinoid receptor 1; TRPV: transient receptor potential vanilloid; LPO: lipid peroxidation; IFNγ: interferon gamma; NF-κB: nuclear factor kappa B; HSP70: heat shock protein 70; iNOS: inducible nitric oxide synthase; ROS: reactive oxygen species; AChE: acetylcholinesterase; BBB: blood-brain barrier; FZD6: frizzled class receptor 6; CTNNB1: catenin beta 1; GSK3B: glycogen synthase kinase 3 beta; CLDN: claudin; CASP3: caspase 3; PKA: protein kinase A; MBP: myelin basic protein; NEFL: neurofilament light chain protein; GPx: glutathione peroxidase; GRx: glutaredoxin; CaMKIIα: calcium/calmodulin-dependent protein kinase II subunit alpha; ATG5: autophagy protein 5; ULK1: UNC51-like kinase 1; LC3B-II: lipidated microtubule-associated protein 1 light chain 3 beta conjugate; NCOA4: nuclear receptor coactivator 4; GluA1: glutamate ionotropic receptor AMPA type subunit 1; HO-1: heme oxygenase 1; Nrf2: nuclear factor erythroid 2-related factor 2; TLR4: Toll-like receptor 4; BDNF: brain-derived neurotrophic factor; TBARS: thiobarbituric acid reactive substances; DCX: doublecortin; GST: glutathione S-transferase; GSSG: glutathione disulfide; Parp1: poly(ADP-ribose) polymerase 1; Gadd45a: growth arrest and DNA-damage-inducible protein 45 alpha; Xrcc1: X-ray repair cross complementing 1; Ogg1:8-oxoguanine DNA glycosylase 1; Ddx5: DEAD-box helicase 5; Ptgs2: prostaglandin-endoperoxide synthase 2; Sat1: spermidine/spermine N1-acetyltransferase 1; Hmox1: heme oxygenase-1; FoxO3: forkhead box O3; FoxP3: forkhead box P3; 4-Hne: 4-hydroxynonenal; Ascl4: achaete-scute family bHLH transcription factor 4; Scl7a11: solute carrier family 7 member 11; FTH1: ferritin heavy chain 1; MMP: matrix metalloproteinase; ALA-D: aminolevulinate dehydratase; T-bet: T-box expressed in T cells; GATA-3: RORγt: retinoic acid-related orphan receptor gamma t; ERβ: estrogen receptor beta; ERRα: estrogen-related receptor alpha; 3-NT: 3-nitrotyrosine; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; γH2AX: gamma H2A histone family member X; H3K9me2: di-methylation of lysine 9 on histone H3 protein; H3K27me3: tri-methylation of lysine 27 on histone H3 protein; THOC5: THO complex subunit 5; PSD95: postsynaptic density protein 95; SYP: synaptophysin; Pin1: Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1; MAPK: mitogen-activated protein kinase; MECP2: methyl-CpG binding protein 2; NLRP3: NOD-like receptor family pyrin domain-containing 3; TRAF6: TNF receptor-associated factor 6

Table 2. Characteristics of included studies using herbal extract.

Herbal extract	Study	Animal model	Sex	Age	Number of groups	Animals per group	Positive control	Route of administration	Treatment timing	Dose	Treatment duration	Main findings	Molecular/biological markers
Anthocyanin-rich extract from Portuguese blueberries	Serra, 2022[58]	prenatal VPA-injected mice	male	3 weeks	4	9–18	none	oral	therapeutic	30 mg/kg	3 weeks	repetitive behavior ↓, social interaction ↑	IL-1β ↓, TNF-α ↓, IL-6 ↓, CD11b ↓, Iba-1 ↓, COX-2 ↓, serotonin ↑, Lactobacillales ↑, Clostridiales ↓
Bacopa monniera (L.) Wettst	Sandhya, 2012[59]	prenatal VPA-injected rat	male	21 days	3	6	none	oral	therapeutic	300 mg/kg	14 days	pain sensitivity ↑, locomotor activity ↓, exploratory activity ↑, anxiety ↓, social interaction ↑	nitrite ↓, GSH ↑, catalase ↑
Cananga odorata essential oil	Zhang, 2023a[60]	prenatal VPA-injected rat	male and female	7 days	5	10–12	none	inhalation	therapeutic	0.83–3.33 mg/L	7 days	anxiety ↓, social interaction ↑, cognition ↑	5-HIAA/5-HT ratio ↑
Crocus sativus (saffron)	Seyedinia, 2023[41]	prenatal VPA-injected rat	male	30 days	4	6	none	intraperitoneal	therapeutic	30 mg/kg	4 weeks	spatial memory ↑, pain sensitivity ↑, balance ability ↑, anxiety ↓	MDA ↓, GSH ↑, CAT ↑
Green tea extract	Banji, 2011[61]	postnatal VPA-injected mice	male and female	14 days	5	12	none	oral	therapeutic	75, 300 mg/kg	4 weeks	negative geotaxis ↓, nociceptive response ↑, mid-air righting ↑, hyperactivity ↓, motor coordination ↑, exploratory activity ↑, anxiety ↓, spatial learning and memory ↑	MDA ↓, damage to Purkinje cell layer ↓
Humulus japonicus	Park, 2021[62]	BTBR mice	male	3 weeks	5	6–13	none	oral	therapeutic	200, 400 mg/kg	6 weeks	self-grooming behavior ↓, social interaction ↑, cognition ↑	IL-1β ↓, IL-6 ↓, CCL2 ↓, Iba-1 ↓, p-NR2B ↓, p-CaMKIIα ↓
Korean red ginseng	Gonzales, 2016[63]	prenatal VPA-injected mice	male	21 days	4	10	none	oral	therapeutic	100, 200 mg/kg	18 days	social interaction ↑, hyperactivity ↓, spatial working memory ↑, repetitive behavior ↓, seizure ↓	n/a
Ocimum basilicum L.	Amini-Khoei, 2025[64]	maternal separation stress in mice	male	2 days	5	15	none	intraperitoneal	therapeutic	20, 40, 60 mg/kg	7 days	spatial learning and memory ↑, passive avoidance memory ↑, social interaction ↑, aggressive behavior ↓	antioxidant capacity ↑, MDA ↓, TLR4 ↓, IL-1β ↓, TNF-α ↓

Passiflora incarnata	Amini, 2023[65]	prenatal VPA-injected rat	male	35 days	8	8	none	oral	therapeutic	30, 100, 300 mg/kg	47 days	vertical activity ↓, repetitive and stereotyped behaviors ↓, anxiety ↓, social interaction ↑, cognition ↑	MDA ↓, CAT ↑, SOD ↑, TAC ↑, neuronal damage ↓
Passiflora ligularis	Al-Radadi, 2024[66]	propionic acid-injected rat	male	n/a	4	10	none	oral	therapeutic	40 mg/kg	3 weeks	social interaction ↑, repetitive and stereotyped behaviors ↓, anxiety ↓	IL-1β ↓, TNF-α ↓, MDA ↓, GSH ↑, SOD ↑, glutamate ↓, serotonin ↑, dopamine ↑, ERK ↓, MBP ↓, Bcl-2 ↑, Bax ↓, caspase 3 ↓, necrosis ↓
Phyllanthus emblica	Gouda, 2024[67]	postnatal VPA-injected mice	male	14 days	3	6	none	oral	therapeutic	100 mg/kg	27 days	motor coordination ↑, hyperactivity ↓, anxiety ↓, social interaction ↑	5-HT1D receptor ↓, 5-HT2A receptor ↓, D2 receptor ↓, NO ↓, MDA ↓, IL-1β ↓, TNF-α ↓, GST ↑, GR ↑
Stigma maydis polysaccharide	Yang, 2024[68]	prenatal VPA-injected rat	male	35 days	5	12	none	dissolve in drinking water	preventive and therapeutic	0.5, 1, 2 g/kg	8 weeks	sociability ↑, repetitive and stereotyped behaviors ↓, hyperlocomotor activity ↓, learning and memory ↑	substance P ↑, enkephalin ↑, VIPs ↑, 5-HT ↑, GI transit time ↓, intestinal Zo-1 and occludin ↑,

Note: 5-HIAA: 5-hydroxyindoleacetic acid; 5-HT: 5-hydroxytryptamine; CCL2: C-C motif chemokine ligand 2; COX-2: cyclooxygenase-2; GR: glucocorticoid receptor; NR2B: N-methyl-D-aspartate (NMDA) receptor subtype 2B; TAC: total antioxidant capacity; VIPs: vasoactive intestinal peptides

Table 3. Characteristics of included studies using traditional formulations.

Traditional formulation	Study	Animal model	Sex	Age	Number of groups	Animals per group	Positive control	Route of administration	Treatment timing	Dose	Treatment duration	Main findings	Molecular/biological markers
Kami-shoyo-san	Guo, 2019a[69]	ovariectomized mice	female	6 weeks	5	8–9	none	oral	therapeutic	74, 222 mg/kg	1 h before behavioral tests	anxiety ↓, social interaction ↑	p-CREB ↑, p-CaMKII α ↑
Kami-shoyo-san	Guo, 2019b[70]	selective type I 5α-reductase inhibitor SKF105111-treated mice	male	5 weeks	4	n/a	none	oral	therapeutic	74, 222 mg/kg	1 h before behavioral tests	social interaction ↑, self-grooming behavior ↓	no change in ALLO level
Qi Bi Anshen decoction	Zhang, 2023b[71]	propionic acid-injected rat	male	6–8 weeks	5	12	none	intragastric	therapeutic	5.04, 10.08, 20.16 g/kg	7 days	social interaction ↑, repetitive and stereotyped behavior ↓, anxiety ↓	IL-1β ↓, IL-6 ↓, TNF-α ↓, neuronal damage ↓, GluN2A ↑, GluN2B ↑, p-GluA1 ↑, MMP9 ↓, BDNF ↑
Yigansan	Fan, 2024[28]	maternal immune activation in mice	male	8 weeks	5	6	none	oral	therapeutic	325, 650, 975 mg/kg	4 weeks	stereotyped behaviors ↓, anxiety ↓, social interaction ↑, cognition and memory ↑	MMP9 ↓, TRAF6 ↓, IL-17A ↓

Note: ALLO: allopregnanolone; GluN2: glutamate ionotropic receptor NMDA type subunit 2

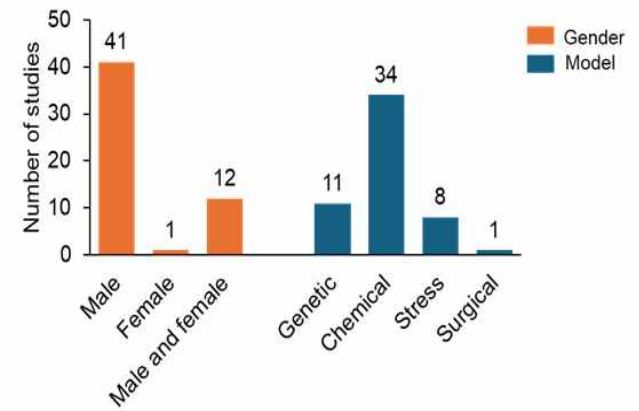


Fig. 3. Gender analysis and types of ASD animal models.

2) Intervention characteristics

Fifty-four studies were classified into three categories according to the intervention type: single compounds, herbal extracts, and traditional formulations (Fig. 4A, Tables 1–3). Among 54 studies, two studies utilized both herbal extracts/herbal formulations and the derived bioactive compounds. Single compounds were the predominant research focus (40 studies, 74.07%), reflecting a modern pharmacological approach that emphasizes isolated active constituents. Single compounds reported in the included studies were divided into five subclasses: polyphenols, terpenoids, cannabinoids, alkaloids, and

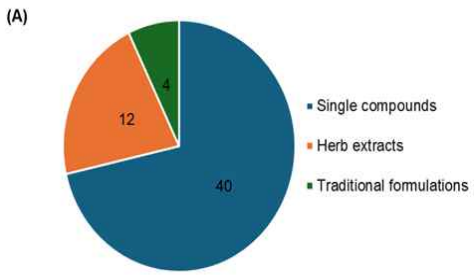
others. The most commonly used compounds are polyphenols, including flavonoids (e.g., apigenin, fisetin, puerarin), phenolic acids (e.g., syringic acid, vanillic acid), stilbenes (e.g., resveratrol), and other polyphenols (e.g., curcumin, quercetin). Resveratrol and cannabidiol (each with five studies, 9.26%) were the most frequently investigated, followed by quercetin (three studies, 5.56%) and fisetin and papaverine (two studies each, 3.70%). Herbal extracts constituted the second most studied category (12 studies, 22.22%), including diverse plant sources such as Portuguese blueberry anthocyanins, *Bacopa monnieri*, *Cananga odorata* essential oil, and Korean red ginseng. Traditional formulations appeared in four studies (7.41%), primarily East Asian traditional medicine prescriptions. Kami-shoyo-san was investigated in two studies (3.70%), whereas Yigansan and Qi Bi Anshen decoctions were examined in one study (1.85%). This distribution suggests a preference for reductionist approaches over traditional whole-herb preparations in preclinical ASD studies.

Seven distinct routes of administration were identified in the 54 studies (Fig. 4B). Oral administration was the most prevalent (26 studies, 48.15%), likely owing to its non-invasive nature, clinical translatability, and high patient compliance in potential therapeutic applications. This preference may reflect the historical context of traditional herbal medicines. Intraperitoneal injection was the second most common route (19 studies, 35.19%), offering advantages, such as increased bioavailability, rapid systemic absorption, and precise dosage control in preclinical research settings. The remaining administration methods—mixing with diet, inhalation, subcutaneous injection, intragastric administration, and dissolution in drinking water—were employed in only 1–2 studies (1.85–3.70%). This diversity in administration routes highlights the different approaches for optimizing compound delivery in animal models of ASD.

Intervention timing strategies were classified into three categories: therapeutic, preventive, and combined preventive-therapeutic approaches (Fig. 4B). The analysis revealed that therapeutic approaches were predominantly employed (43 studies, 79.63%), followed by combined preventive-therapeutic approaches (seven studies, 12.96%) and purely preventive approaches (five studies, 9.26%). Notable patterns emerged when examining the intervention timing in relation to the animal model type. Genetic models were used exclusively in therapeutic intervention studies, reflecting their utility for evaluating treatments for established ASD phenotypes. Chemically induced models showed greater diversity in terms of timing strategies, with a small subset employing preventive approaches. Stress-induced models exhibited the broadest range of intervention timing approaches. These results suggest that research has primarily focused on treating established ASD symptoms rather than preventing their development.

Treatment durations across the 54 studies were categorized into four intervals: acute (single dose), short-term (1–7 days), medium-term (8–28 days), and long-term (>28 days) (Fig. 4B). Medium-term interventions were predominant (28 studies, 51.85%), reflecting the balance between sufficient intervention time and practical research parameters. Short-term interventions were employed in 11 studies (20.37%), which often targeted specific developmental windows or evaluated immediate therapeutic responses. Long-term interventions were described in 9 studies (16.67%), allowing for the assessment of sustained effects and better approximation of clinical treatment durations. Acute administration was performed in six studies (11.11%), primarily to examine the immediate pharmacological effects on behavioral outcomes. The limited number of long-term studies may present challenges in understanding the sustained efficacy of herbal interventions in ASD.

Dosage distribution analysis was conducted for 48 studies that reported doses in mg/kg (Fig. 4B). Three dosage categories were established: low (≤10 mg/kg), medium (>10–50 mg/kg), and high (>50 mg/kg). Medium doses were most frequently used (26 studies, 54.17%), followed by high doses (20 studies, 41.67%) and low doses (10 studies, 20.83%). Some studies used multiple dose ranges and were accordingly counted in multiple categories. Medium doses were predominant across all treatment duration categories (acute, short, medium, and long term). Short-term administration studies showed a relatively higher proportion of low-dose usage than other duration categories, although the sample size was insufficient to establish definitive correlations.



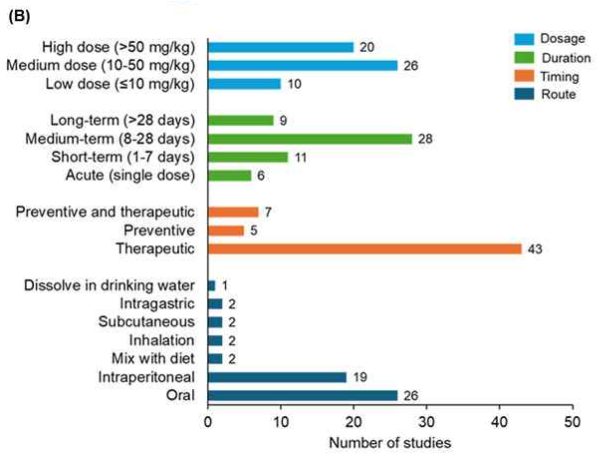


Fig. 4. Treatment characteristics of included studies. (A) Intervention categories. (B) Treatment routes, timing, duration, and dosages.

3) Behavioral assessments

The behavioral tests employed across the 54 studies were classified into six categories, with most studies utilizing multiple assessment types to comprehensively evaluate intervention effects (Fig. 5). Social behavior tests were most frequently employed (42 studies, 77.78%), primarily three-chamber tests, social interaction tests, and sociability assessments, to evaluate core social deficits in ASD. Anxiety-like behavior tests constituted the second most prevalent category (40 studies, 74.07%), featuring open-field tests, elevated plus maze tests, and light-dark box paradigms. Repetitive behavioral, learning, and memory tests were performed in 25 studies (46.30%). Repetitive behavior assessments predominantly employ marble burying tests and self-grooming quantification to evaluate restricted, repetitive behavioral patterns characteristic of ASD. Learning and memory evaluations frequently utilize novel object recognition tests and the Morris water maze paradigm. Motor function tests were performed in ten studies (18.52%), primarily using the rotarod test to assess motor coordination and balance. Sensory tests were the least common (eight studies, 14.81%), including hot plate tests, prepulse inhibition paradigms, and nociceptive assessments. This distribution of assessment methods reflects a primary focus on the core social and repetitive behavioral domains of ASD, although limited attention to sensory abnormalities presents a notable gap in comprehensive phenotypic evaluation.

4) Brain regional analysis

Analysis of the brain regions investigated across 54 studies revealed distinct regional focus patterns (Fig. 6). The hippocampus was the most frequently studied region (23 studies, 42.59%) followed by the cerebellum (15 studies, 27.78%). Whole-brain analyses were conducted in nine studies (16.67%), providing broader neurobiological insights but lacking region-specific details. Various cortical regions were examined, including the prefrontal and general cortices (6 studies, 11.11%), cerebral cortex (4 studies, 7.41%), medial prefrontal cortex, and frontal cortex (3 studies, 5.56%). The striatum was investigated in 6 studies (11.11%), highlighting the importance of subcortical structures. The amygdala was examined in only two studies (3.70%). This regional distribution indicates a particular research interest in the hippocampus and cerebellum, with a secondary focus on the cortical regions and striatum across preclinical ASD studies involving herbal interventions. The limited investigation of the amygdala suggests a potential gap in understanding the effects of herbal medicines on social-emotional processing circuits in ASD models.

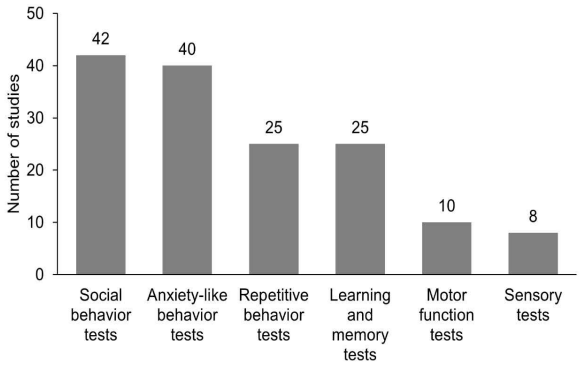


Fig. 5. Behavioral test categories distribution.

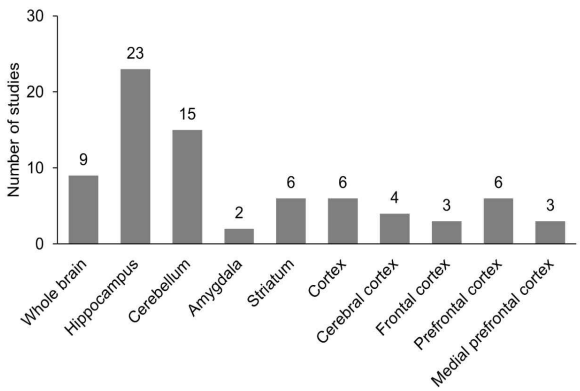


Fig. 6. Brain regional analysis.

5) Molecular markers and mechanisms of action

Analysis of the 54 studies revealed six principal mechanisms through which herbal medicines exert therapeutic effects in ASD models (Fig. 7 and Table 1). Antioxidant activity was the most prevalent mechanism (26 studies, 48.15%), characterized by decreased malondialdehyde (MDA) levels and increased levels of

antioxidant enzymes (GSH, CAT, and SOD). Anti-inflammatory effects constituted the second most common mechanism (21 studies, 38.89%), evidenced by downregulation of pro-inflammatory cytokines (TNF-α, IL-1 β, IL-6) and inflammatory mediators.

Neurotransmitter regulation and neuroplasticity enhancement were observed in 8 studies (14.81%). Neurotransmitter regulation involves increased serotonin and GABA levels, with concurrent decreases in glutamate levels. Neuroplasticity enhancement is indicated by elevated levels of BDNF, p-CREB, and synaptic proteins. Anti-apoptotic effects were documented in six studies (11.11%), as reflected by reduced caspase-3 and Bax expression, and increased Bcl-2 levels.

Less frequently observed were improvements in mitochondrial function (four studies, 7.41%) and gut-brain axis modulation (three studies, 5.56%). Multiple studies have reported the simultaneous modulation of several pathways, rather than operating through singular mechanisms. This mechanistic profile suggests that herbal interventions may address the multifaceted pathophysiology of ASD through complementary pathways; however, certain mechanisms remain underexplored.

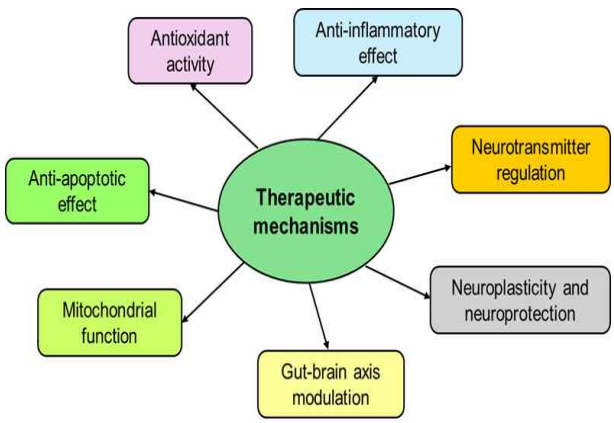


Fig. 7. Mechanisms of action of herbal medicines.

6) Safety profile

Analysis of adverse effect reporting across 54 studies revealed significant gaps in safety monitoring (Fig. 8). Most studies (44 studies, 81.48%) provided no information regarding adverse effects, indicating a substantial limitation in safety reporting. Nine studies (16.67%) reported no adverse effects during herbal medicine administration, whereas only one (1.85%) documented adverse effects. A single study that reported adverse effects investigated alpha-glycosyl isoquercitrin administered at doses of 0.25% and 0.5% over 77 days in a postnatal LPS-injected rat model[18]. The reported adverse effects include decreased body weight, reduced food and water consumption, and a mortality rate of 20-30%. This lack of safety monitoring presents a significant concern for the translational value of preclinical herbal medicine research in ASD.

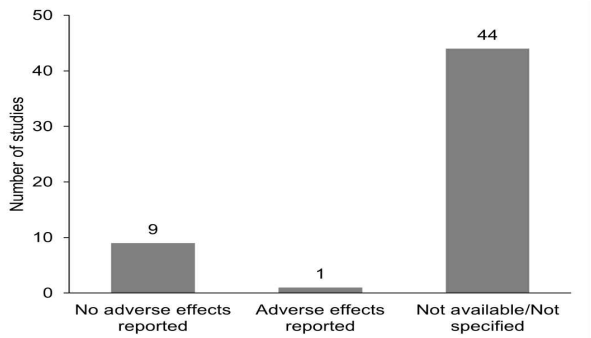


Fig. 8. Adverse effects reporting.

7) Use of positive control drugs

Among the 54 included studies, the use of FDA-approved drugs for ASD in animal models was limited. Only one study used risperidone, whereas another study incorporated aripiprazole as a positive control drug[24,56]. The doses of natural compounds used in these studies were generally higher than those of positive control drugs. Ligustilide (10 mg/kg) and risperidone (2.5 mg/kg) were reported in one study to have comparable effects on social interaction, repetitive behaviors, and motor function, with ligustilide (30 mg/kg) showing superior efficacy over positive controls[56]. In another study, genistein (80 mg/kg) exhibited similar effects on ASD behavior and biochemical markers to aripiprazole (5 mg/kg)[24].

4. Quality assessment of the included studies

The outcomes of the quality assessment are presented in Table 4. Fifty-four studies described the study design with details about experimental animals and procedures, as well as the measurement of experimental outcomes compared with the control groups. Fifty-four studies clearly demonstrated the study objectives and generalizability of the findings. Thirty studies (56%) did not include any statements regarding blinding in the experimental procedures, outcome evaluation, or data analysis. Fifty-four studies had sufficient information about sample size, randomization, and independent replications. Only one study (2%) did not provide an accurate summary in the abstract. Fifty-four studies provided statistical methods, but only one study (2%) clearly described the methods used to check the assumptions of the statistical

tests. Eight studies (15%) described sufficient background and explained the reasons for animal model selection. Fifty-two studies (96%) provided ethical statements as well as housing and husbandry conditions. None of the studies (0%) provided a detailed description of the methods used to reduce animal suffering. Twenty studies (37%) interpreted the results with comments on the study limitations. Forty-nine studies (91%) registered protocols for animal experiments. Thirty-one studies (57%) provided statements regarding the data availability.

Table 4. Quality assessment of the included study.

Reference	Item																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Al-Amin, 2015[39]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	2	2	1	0
Alhusain, 2024[26]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	1	2	1	2
Al-Radadi, 2024[66]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	1	2	2	1	0
Amini, 2023[65]	2	1	1	1	0	2	1	2	2	1	2	1	2	0	2	0	1	2	0	2
Amini-Khoei, 2025[64]	2	1	1	1	1	2	1	2	2	1	1	1	2	2	2	0	2	2	1	2
Anand, 2024[42]	2	1	1	1	1	2	1	2	2	1	1	1	2	2	2	0	2	2	1	2
Bakheet, 2017[31]	2	1	1	1	0	2	1	2	2	1	1	2	2	2	2	0	1	2	1	0
Banji, 2011[61]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	1	2	1	0
Bhandari, 2017[34]	2	1	1	1	1	2	1	2	2	1	1	2	2	2	0	0	1	2	1	0
Chen, 2025[40]	2	1	1	1	0	2	1	2	2	1	1	2	2	2	2	0	1	2	1	0
Erten, 2021[43]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	2	2	0	0
Fan, 2024[28]	2	1	1	1	2	2	1	2	2	1	1	1	2	2	2	1	2	2	1	0
Farzan, 2023[38]	2	1	1	1	1	2	1	2	2	1	1	1	2	2	2	0	1	2	1	2
Fujita, 2020[55]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	2	2	1	2
Gonzales, 2016[63]	2	1	1	1	1	2	1	2	2	1	1	1	2	2	2	1	1	2	1	0
Gouda, 2024[67]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	1	1	2	1	2
Guo, 2019a[69]	2	1	1	1	1	2	1	2	2	1	2	1	2	2	2	0	1	2	1	0
Guo, 2019b[70]	2	1	1	1	1	2	1	2	2	1	1	1	2	2	2	0	1	2	1	2
Jayaprakash, 2021[21]	2	1	1	1	0	2	1	2	2	1	1	2	2	2	2	0	2	2	1	0
Jayaprakash, 2024[19]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	2	2	1	2
Jiang, 2024[27]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	1	1	2	1	0
Kaplan, 2017[46]	2	1	1	1	1	2	1	2	2	1	1	2	2	2	1	0	1	2	0	0
Karimi, 2023[57]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	2	2	1	2
Khalaj, 2018[25]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	1	1	2	1	0
Kumar, 2025[24]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	2	2	1	2
Li, 2024[48]	2	1	1	1	1	2	1	2	2	1	1	1	2	2	2	0	1	2	1	2
Liu, 2024[51]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	1	2	1	2
Luhach, 2021a[52]	2	1	1	1	1	2	1	2	2	1	1	1	2	2	2	0	1	2	1	2
Luhach, 2021b[53]	2	1	1	1	1	2	1	2	2	1	1	1	2	2	2	0	1	2	1	2
Mahmoudian, 2024[36]	2	1	1	1	1	2	1	2	2	1	1	1	2	2	2	0	2	2	1	2
Mallan, 2023[37]	2	1	1	1	1	2	1	2	2	1	1	1	2	2	2	1	1	2	1	0
de Mattos, 2020[29]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	1	1	2	1	2
Mehra, 2023[22]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	1	1	2	1	2
Mehra, 2024[23]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	1	2	1	2
Mehta, 2021[20]	2	1	1	1	1	2	1	2	2	1	1	1	2	2	2	0	2	2	1	0
Moghaddam, 2023[30]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	1	2	1	2
Okano, 2022[18]	2	1	1	1	1	2	2	2	2	1	1	1	2	0	2	1	1	2	0	0
Park, 2021[62]	2	1	1	1	1	2	1	2	2	1	1	2	2	2	2	0	1	2	0	2
Patra, 2020[49]	2	1	1	1	2	2	1	2	2	1	1	1	2	2	2	0	1	2	1	2
Pedrazzi, 2025[47]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	2	2	1	2
Pragnya, 2014[54]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	1	2	1	0
Sandhya, 2012[59]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	1	2	1	0
Serra, 2022[58]	2	1	1	1	1	2	1	2	2	1	2	1	2	2	2	1	1	2	1	2
Seyedinia, 2023[41]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	2	2	1	2
Shahrbabaki, 2023[35]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	1	2	1	2
Sherawat, 2024[44]	2	1	1	1	1	2	1	2	2	1	1	1	2	2	2	1	2	2	1	2
Staben, 2023[50]	2	1	1	1	1	2	1	2	2	1	1	2	2	2	2	0	2	2	1	2
Xie, 2018[32]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	2	2	1	0
Xiong, 2023[45]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	0	1	2	1	2
Yang, 2024[68]	2	1	1	1	1	2	1	2	2	1	1	1	2	2	2	0	2	2	1	2
Zeng, 2024[33]	2	1	1	1	0	2	1	2	2	1	0	1	2	2	2	0	2	2	1	0
Zhang, 2023a[60]	2	1	1	1	0	2	1	2	2	1	1	1	2	2	2	1	1	2	1	0
Zhang, 2023b[71]	2	1	1	1	1	2	1	2	2	1	1	1	2	2	2	0	1	2	1	2
Zhou, 2024[56]	2	1	1	1	1	2	1	2	2	1	1	2	2	2	2	0	1	2	1	0
the percentage of "0"	0	0	0	0	56	0	0	0	0	0	2	0	0	4	2	76	0	0	9	43

the percentage of "1"	0	100	100	100	41	0	98	0	0	100	93	85	0	0	2	24	63	0	91	0
the percentage of "2"	100	0	0	0	4	100	2	100	100	0	6	15	100	96	96	0	37	100	0	57

Item: (1) study design, (2) sample size, (3) inclusion and exclusion criteria, (4) randomization, (5) blinding, (6), outcome measures, (7) statistical methods, (8) experimental animals, (9) experimental procedures, (10) results, (11) abstract, (12) background, (13) objectives, (14) ethical statement, (15) housing and husbandry, (16) animal care and monitoring, (17) interpretation/scientific implications, (18) generalizability/translation, (19) protocol registration, and (20) data access. Scoring: "2", clearly sufficient; "1", possibly sufficient; "0", clearly insufficient.

Discussion

This scoping review provides a comprehensive analysis of preclinical evidence on the use of herbal medicines and natural compounds in the treatment of ASD. Our findings revealed several patterns in research methodology, intervention strategies, and therapeutic mechanisms that could inform the interpretation of ASD treatment approaches.

Temporal distribution analysis demonstrated a notable acceleration in research activity, with 72.22% of the studies published within the most recent 5-year period (2021–2025). This surge likely reflects the growing recognition of the potential of herbal interventions to address the multifaceted pathophysiology of ASD alongside the increasing standardization of animal models. However, this recency also indicates that the field remains at a relatively nascent stage of development, suggesting caution in extrapolating these findings to clinical applications. The geographical distribution of the studies revealed a pronounced regional imbalance, with Asian countries contributing 83.33% of all the research. This pattern likely stems from the strong cultural and historical foundations of traditional medicine in these regions, particularly India, China, and Iran, which collectively represented 62.97% of the included studies. Cultural context significantly influences approaches to ASD treatment and intervention responses, highlighting the necessity for expanding research across diverse cultural settings. The limited representation of Western countries (9.26%) suggests a need for greater global collaboration to integrate diverse pharmacological approaches and ensure that the findings translate across different healthcare paradigms.

Our analysis of animal models revealed a clear preference for chemically induced models (62.96%), particularly VPA-induced models (42.59%). This prevalence likely stems from the relative ease of implementation and established face validity in replicating core ASD behavioral features[10]. However, considering the heterogeneous etiology of ASD, overreliance on a single model may limit the generalizability of the findings. The integrated use of diverse animal models is essential for a comprehensive understanding of the complex pathophysiological mechanisms underlying ASD. The underrepresentation of genetic (20.37%) and stress-induced (14.81%) models is noteworthy, as these models may better capture certain aspects of the complex pathophysiology of ASD. Furthermore, 75.93% of studies used male animals exclusively, thereby signifying a striking sex imbalance, which represents a significant methodological limitation. Neurobiological differences in ASD between sexes can influence treatment responses. Female patients with ASD often present with symptom patterns different from those observed in male patients, and neuroanatomical and neurochemical differences may affect responses to specific interventions[72]. This sex gap in preclinical research parallels the historic male bias in clinical ASD research and contradicts the increasing recognition of ASD in female patients.

Intervention analysis revealed a predominance of isolated compounds (68.52%) over herbal extracts (22.22%) and traditional formulations (7.41%). This emphasis on single compounds aligns with the reductionist approach of modern pharmacology, facilitating mechanistic understanding while potentially overlooking the synergistic effects inherent to whole herbs or traditional formulations. Multicomponent herbal preparations may modulate multiple pharmacological targets simultaneously, potentially offering broader efficacy and fewer side effects than single compounds.

The preference for oral administration (48.15%) demonstrates appropriate consideration for clinical translatability, as it is most analogous to typical human consumption patterns. The intervention timing analysis indicated a strong preference for therapeutic strategies (79.63%) over unknown "preventive" approaches (9.26%). However, the term "preventive" should be understood not as preventing ASD occurrence, but as an early intervention during critical neurodevelopmental periods to mitigate symptom manifestation or severity. Brain overgrowth during the first 1–2 years of life in patients with ASD represents a critical window for intervention in developmental trajectories. Early interventions for children with ASD aged <3 years provide opportunities to positively influence symptomatic pathways by leveraging heightened neuroplasticity during this period[73]. Additionally, the prevalence of medium-term interventions (51.85%) reflects a practical compromise between research feasibility and

clinical relevance; nonetheless, the limited proportion of long-term studies (16.67%) suggests a restricted understanding of sustained treatment effects—a critical consideration, given the lifelong impact and chronic nature of ASD. Patients require lifelong support, underscoring the importance of investigating the effects of long-term interventions.

Behavioral assessment patterns demonstrated appropriate emphasis on social behavior (77.78%) and anxiety-like behavior (74.07%), aligning with the core and common comorbid symptoms of ASD as defined in the DSM-5 and ICD-11[74]. However, the relatively low rate of assessment of repetitive behaviors (46.30%) represents a notable imbalance. Restricted and repetitive behaviors extend beyond mere symptoms to serve as important outcome measurements for intervention efficacy and require systematic evaluation[75]. The limited attention paid to sensory abnormalities (14.81%) represents an even more concerning gap. Considering the explicit inclusion of sensory abnormalities in DSM-5 diagnostic criteria for ASD, this gap is particularly troubling. Sensory hypersensitivity or hyporesponsiveness is reported in approximately 90% of individuals with ASD and significantly affects social interaction abilities, anxiety levels, and overall quality of life[76]. Sensory processing abnormalities may constitute one of the core pathophysiological mechanisms underlying ASD[77]. Future studies should adopt more comprehensive behavioral assessment batteries that encompass the full spectrum of ASD manifestations, with enhanced measures related to sensory processing.

Regional brain analysis revealed focused attention on the hippocampus (42.59%) and cerebellum (27.78%), reflecting an increased recognition of the roles of these structures in ASD pathophysiology beyond the traditional focus on fronto-striatal circuits[78]. Cerebellar abnormalities influence social cognition and language development in patients with ASD. However, the limited investigation of the amygdala (3.70%) represents a notable gap, given its established role in social-emotional processing—a core deficit in ASD. The amygdala plays a crucial role in processing social stimuli and regulating emotions, which are directly related to core ASD symptoms[79]. Future research should adopt more balanced regional analyses and incorporate advanced techniques, including circuit-specific optogenetics, to elucidate region-specific treatment effects.

Mechanistic analyses identified antioxidant (48.15%) and anti-inflammatory (38.89%) activities as the predominant therapeutic pathways, which aligns with recent research on the roles of oxidative stress and neuroinflammation in ASD pathophysiology[80]. Systematic analyses have documented increased levels of oxidative stress biomarkers and decreased antioxidant defense capabilities in patients with ASD[2]. Additionally, inflammatory imbalances characterized by elevated levels of pro-inflammatory cytokines (IL-6, TNF-α) and reduced levels of anti-inflammatory cytokines (IL-10) have been observed in the serum of children with ASD[81]. Nevertheless, relatively limited research on neuroplasticity enhancement (14.81%), neurotransmitter regulation (14.81%), and gut-brain axis modulation (5.56%) represents a notable gap. Abnormal expression of synaptic proteins and impaired neuroplasticity have been observed in patients with ASD and represent important therapeutic targets[82]. Furthermore, gut microbiota dysbiosis is closely associated with behavioral and neurological alterations in ASD, suggesting that the gut-brain axis is a potential therapeutic target[83]. The comparative scarcity of studies investigating the effects of herbs on these domains represents a critical area for future research. The multi-mechanistic action exhibited by many herbal interventions may offer potential advantages over conventional pharmaceuticals that target single receptors or pathways in the treatment of complex neurodevelopmental disorders[84]. Multi-target drugs may prove to be more effective than single-target approaches for complex neuropsychiatric conditions such as ASD, highlighting the important clinical implications of the multifaceted action mechanisms of herbal medicines and natural compounds.

Safety profiles were inadequately reported, with 81.48% of studies providing no information on adverse effects. This concerning gap raises ethical concerns and limits the translational value of our findings. While herbal medicines are often presumed to be safe based on traditional usage, rigorous preclinical safety assessment remains essential, given the evidence of herb-drug interactions and variability in phytochemical compositions. The lack of systematic safety reporting is a significant barrier to clinical translation and requires immediate attention in future research.

Among the compounds reviewed, resveratrol, cannabidiol, and quercetin exhibit the most consistent therapeutic effects in multiple ASD models. These compounds were particularly efficacious in improving social interaction deficits and reducing repetitive behaviors, which are core symptoms inadequately addressed by current

FDA-approved medications[85]. The observed multi-pathway modulation by these compounds suggests potential advantages over single-target pharmaceuticals, particularly when considering the complex pathophysiology of ASD. The established safety profiles of these compounds under other conditions further support their clinical potential; nevertheless, ASD-specific safety data remain limited. Translating these findings into clinical practice requires careful consideration of optimal formulations, dosages, and treatment durations, particularly in pediatric populations. Additionally, the potential of herbal interventions as adjunct therapies to established behavioral interventions should be further investigated to improve the treatment outcomes through complementary mechanisms.

Our review revealed that only a limited number of studies have incorporated FDA-approved drugs (i.e., risperidone and aripiprazole) for ASD as positive control drugs[24,56]. The use of clinically validated medications as reference drugs is crucial for establishing the comparative efficacy of herbal interventions. Without such comparisons, it is difficult to determine whether the effects of herbal medicines are similar to or superior to those of available pharmacological treatments. The natural compounds used in the included studies were typically administered at higher doses, producing effects that were either comparable to or moderately more effective than those of positive control drugs. Future research with appropriate dosage comparisons should include FDA-approved drugs as positive controls to enhance the reliability of the findings. The combination of conventional drugs and herbal medicines may offer a promising therapeutic approach for ASD treatment.

This review summarizes the preclinical evidence on the therapeutic potential of herbal medicines in the treatment of ASD, laying the foundation for their translation into clinical research and practice. In recent years, several clinical investigations have examined the efficacy of herbal medicines in pediatric patients with ASD, particularly focusing on traditional formulations reported to alleviate behavioral symptoms and improve sleep quality[9,13]. Although the number of high-quality randomized controlled trials remains limited, the existing findings offer encouraging evidence supporting the translational relevance of preclinical studies. To further bridge the gap between animal research and clinical application, future studies should focus on systematic evaluation of pharmacokinetics, safety profiles, and dose-response relationships of promising herbal candidates. Notably, our review reveals that in vivo studies employing traditional multi-herbal prescriptions remain relatively scarce compared to those investigating single compounds. Given that traditional formulations consist of multiple herbs with potential synergistic effects, future preclinical research should extend beyond single-compound investigations and incorporate standardized multi-herbal formulations to enhance clinical applicability. Furthermore, integrating modern analytical approaches, such as network pharmacology, molecular docking, transcriptomics, and metabolomics, may facilitate the identification of bioactive constituents and molecular targets, thereby strengthening the scientific basis for the clinical translation of herbal medicines in ASD treatment.

Conclusion

This review documents a substantial increase in preclinical evidence supporting the use of herbal medicines in ASD treatment, particularly the consistent efficacy of resveratrol, cannabidiol, and quercetin in multiple animal models. The use of these compounds leads to specific improvements in social interaction deficits and repetitive behaviors via the modulation of oxidative stress markers, inflammatory cytokines, and synaptic plasticity factors. Despite these promising results, critical methodological limitations, such as the predominance of male-only studies, overreliance on VPA-induced models, insufficient safety profiling in most studies, and limited investigation of neuroplasticity and gut-brain axis mechanisms, require attention.

Future investigations should prioritize comparisons between whole-herb extracts and isolated compounds to assess their synergistic effects, implement appropriate sex-balanced study designs, and evaluate dose-dependent responses across extended treatment timeframes to address the chronic nature of ASD. Standardized safety reporting protocols and expanded mechanistic investigations beyond the antioxidant and anti-inflammatory pathways could substantially improve the translational value of these findings for clinical applications. Additional focus on understudied but promising mechanisms, particularly gut-brain axis modulation and neuroplasticity enhancement, may reveal novel therapeutic targets for ASD interventions.

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