



Network Pharmacology and Molecular Docking Reveal Multi-Target Mechanisms of Luteolin Against Autism Spectrum Disorder

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ABSTRACT

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition influenced by genetic, metabolic, and neuroinflammatory mechanisms. Luteolin, a dietary flavonoid with anti-inflammatory and neuroprotective properties, has recently gained attention as a potential adjunct therapy for ASD. However, its molecular mechanisms remain insufficiently explored. This study employed a network pharmacology framework to identify putative molecular targets of luteolin in ASD. Targets were predicted using SwissTargetPrediction and intersected with ASD-related genes retrieved from GeneCards (relevance score >1). Protein-protein interaction (PPI) networks were constructed using STRING and analyzed via Cytoscape and CytoHubba to determine key hub genes. Functional enrichment was performed using GO and KEGG analyses. Representative hub proteins including CA4, MET, DRD4, AKT1, and IGF1R from major ASD-related pathways were further validated through CB-Dock molecular docking. A total of 39 overlapping targets were identified. Hub nodes (AKT1, SRC, ESR1, GSK3B, PTGS2, MMP9, PARP1, IGF1R, AR, ESR2) were strongly enriched in pathways central to ASD pathology, including nitrogen metabolism, dopaminergic synapse, HIF-1 signaling, adherens junction, and PI3K-Akt signaling. Molecular docking confirmed strong ligand-protein interactions, with binding energies ranging from -7.7 to -9.8 kcal/mol, indicating favorable affinity particularly with AKT1 (-9.8 kcal/mol), DRD4 (-8.9 kcal/mol), and IGF1R (-8.3 kcal/mol). This integrative analysis suggests that luteolin exerts multitarget effects relevant to ASD through modulation of synaptic signaling, oxidative stress, inflammatory pathways, and excitatory/inhibitory neurotransmission. These findings provide mechanistic support for luteolin as a potential complementary therapeutic agent for ASD and justify further clinical investigations.

Keywords: Autism, Luteolin, Network Pharmacology, Molecular Docking

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disease that affects social interaction, language and communication skills, and repetitive behaviors [1]. Research indicates that the global prevalence of ASD has significantly increased over time, with a median prevalence of 100/10,000 across regions [2]. The cause of persistent autistic symptoms are multiple, coming from a complex interaction of genetic and environmental factors that begin early in development. Genetic mutations contribute for a substantial number of instances, although environmental factors like as maternal diabetes, infections, pollution exposure, and advanced parental age are also considered important risk factors that might combine with a person's genetic predisposition. No single explanation has been found, and the combination of these variables is thought to create the brain changes observed in persons with autism [3]. The cause of ASD has yet to be discovered. The most common therapeutic approaches are pharmacological intervention, comprehensive education, rehabilitation training, sensory integration, and nutrition therapy. Unfortunately, none of these interventions have been scientifically proven to improve the underlying characteristics of ASD.

Herbal treatments, a type of complementary and alternative medicine (CAM), are increasingly popular as therapeutic options for children with ASD due to their lower risk of adverse drug effects [4]. The number of RCT studies on herbal medicine equals that of conventional therapy, suggesting that herbal medicine is widely prescribed and accepted around the world [5]. In recent years, the number of studies concentrating on the advancement of network pharmacology in Chinese medicine has increased significantly. Based on these biological datasets and clinical trial results, researchers can utilise systems biology to study the network connection between "herbs-compounds-proteins/genes-disease" and scientifically explain how herbal medicines function on certain disorders. The importance of network pharmacology stems from the ability to generate fresh ideas and approaches to drug discovery. As a result, biological systems,

medications, and diseases can be linked in a dependable network, promoting herbal medicine innovation [6].

Flavonoids are considered generally safe and are being discussed as possible treatment of central nervous system disorders that may involve brain inflammation in response to environmental triggers [7-11] is a natural flavonoid found in foods including carrots, apples, cabbage, and several medicinal plants. It has a variety of pharmacological actions, including anticancer, anti-inflammatory, and neuroprotective activities [12]. Luteolin's structure determines its antioxidant capability and resistance to oxidation through metal ion chelation [13-15].

Luteolin has shown promise in preclinical and small-scale clinical investigations as a possible supplemental treatment for autism spectrum disorder (ASD) symptoms, owing to its anti-inflammatory and antioxidant characteristics. However, additional large-scale clinical trials are required to thoroughly assess its safety and efficacy in humans [16-17]. These clinical and animal model studies demonstrated that Luteolin has neuroprotective properties, suggesting that it may be a beneficial therapeutic agent for treating ASDs.

Glutamate excitotoxicity is one of the neurochemical etiological pathways of autism spectrum disorder (ASD) caused by an imbalance in excitatory and inhibitory neurotransmission, resulting in high glutamate levels and subsequent neuronal damage. This imbalance could be caused by dysregulated glutamate transporters, neuroinflammation, or oxidative stress, all of which contribute to autism's primary symptoms. According to research, autistic patients have higher glutamate concentrations and altered glutamate/GABA levels, which is corroborated by animal models and post-mortem tests [18].

In this study, we employed a network pharmacology technique, followed by molecular docking analysis, to determine the possible targets, and pharmacological mechanism of Luteolin in ASD treatment. This would provide a

vital and basic information for future study into the pharmacological mechanism of luteolin

METHODS

Pharmacokinetics Screening of Luteolin

Swiss ADME, ADMET Predictor databases were used to evaluate the pharmacokinetic properties of luteolin through SMILES, with a focus on key factors such as absorption, distribution, metabolism, and excretion (ADME). The Protox-3 webserver was used to define toxicological endpoints, including hepatotoxicity, carcinogenicity, immunotoxicity, and cytotoxicity, as well as median lethal doses (LD₅₀).

Target Prediction and Network Pharmacology of Luteolin in Autism

The SwissTargetPrediction database was utilized to predict the potential targets of Luteolin through SMILES. Additionally, potential targets for Autism were gathered from the GeneCards database using the keyword Autism. From this, we selected Autism related genes with a correlation score greater than 1. The overlapping targets, Luteolin and Autism, were identified using a Venn diagram, which represented the potential targets of Luteolin in the treatment of Autism

Construction of Protein-Protein Interaction (PPI) Network

To analyze the functional interactions of proteins related to Luteolin and Autism the STRING database was used to build a protein-protein interaction (PPI) network. A confidence score threshold of ≥ 0.4 was applied to identify relevant targets, with Homo sapiens selected as the species of interest. The network was then visualized and further analyzed using Cytoscape software (version 3.8.1). To refine the analysis Cytoscape plug-ins were employed to identify key core targets involved in Luteolin against Autism which were used to construct a regulatory network.

GO and KEGG Pathway Enrichment Analysis

Gene Ontology (GO) functional and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed to explore the underlying mechanisms and pathways associated with Luteolin in the treatment of Autism. These analyses were conducted using the ShinyGO database for the 39 common targets. The criteria for enrichment analysis included a p-value FDR cutoff of 0.05 covering the categories of biological process (BP), cellular component (CC), molecular function (MF), and KEGG pathways.

Molecular Docking verification

To assess the potential binding interactions between the Luteolin with its core targets in autism, molecular docking was performed using the CB-Dock online server (AutoDock Vina-based tool) to explore their binding interactions. Target protein structures were selected based on a comprehensive evaluation of resolution and release time from the Protein Data Bank (PDB). The chemical structures of the Luteolin as SDF files was retrieved from the PubChem database.

Results.

Pharmacokinetic Prediction of Luteolin

Luteolin structures, bioavailability radar plot, and main targets are shown in Figure 1. The bioavailability radar (pink area exhibits optimal range of particular property) is shown in Figure 1b. Luteolin exhibits moderate lipophilicity (LIPO), which supports its ability to cross cell membranes and reach target sites. Its moderate flexibility (FLEX = flexibility as per rotatable bonds) and moderate molecular size (SIZE= size as molecular weight), enhances interactions with various biological targets and good permeability respectively. Luteolin shows moderate polarity (POLAR) and presence of unsaturation (INSATU) suggests have reactive sites for its biological activity. Luteolin shows a moderate degree of insolubility (INSOLU). The pie chart in Figure1c. illustrates different protein targets for Luteolin with the major portion as enzymes, followed by cytochrome P450 proteins and lyases. Smaller proportions are attributed to kinases,

oxidoreductases, and proteases. The physicochemical properties of Luteolin are presented in Table 1. Luteolin with molecular formula C₁₅H₁₀O₆ has a molecular weight of 286.24 g/mol and a high gastrointestinal absorption potential, making it suitable for oral administration. It contains 21 heavy atoms, with 16 aromatic ones, and has a low fraction of sp³-hybridized carbons. The compound exhibits moderate polarity, as indicated by its topological polar surface area (TPSA) of 111.13 Å², which may aid in its solubility and absorption. It also inhibits key drug-metabolizing enzymes, including CYP1A2, CYP2D6, and CYP3A4. It is unlikely to cross the blood-brain barrier, limiting central nervous system effects, and it has very low skin permeability, suggesting it is unsuitable for topical use. Its molar refractivity of 76.01 suggests moderate polarizability. ADMET and toxicity predictions by Protox-3 of Luteolin, as shown in Table 2, indicate a safe profile. Luteolin, with a predicted LD₅₀ of 3919 mg/kg, demonstrates a relatively safe toxicity profile, showing negative results for mutagenicity, carcinogenicity, and hERG channel blocking, though it is classified as a skin sensitizer. It is inactive for hepatotoxicity, neurotoxicity, and cardiotoxicity, but shows potential activity for nephrotoxicity and respiratory toxicity. Luteolin interacts minimally with various molecular receptors, indicating a low likelihood of disrupting essential biological processes.

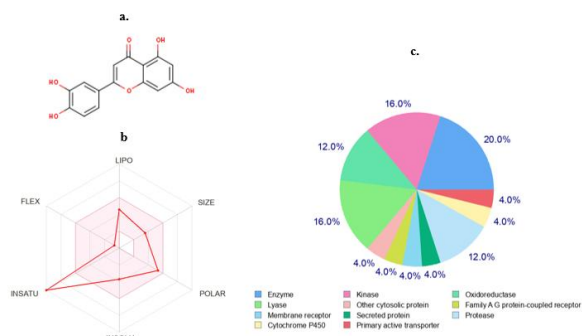


Figure 1 a. Structures, b. Bioavailability Radar and c. top molecular targets of Luteolin

Table 1 - Physicochemical and Pharmacokinetics properties of Luteolin

Properties	Luteolin
Formula	C ₁₅ H ₁₀ O ₆
Molecular weight	286.24
Num. heavy atoms	21
Num. arom. heavy atoms	16
Fraction Csp ³	0
Num. rotatable bonds	1
Num. H-bond acceptors	6
Num. H-bond donors	4
Molar Refractivity	76.01
GI absorption	High
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	Yes
Log Kp (skin permeation)	-6.25 cm/s
TPSA (Topological polar surface area)	111.13 Å ²

Table 2 - ADMET and toxicity prediction by Protox-3 of Luteolin

Properties		Luteolin	
Predicted LD ₅₀		3919mg/kg	
AMES toxicity		Negative	
Carcinogens		Negative	
Ames Mutagenicity		Negative	
Skin Sensitisation		Sensitizer	
hERG (hERG Blockers)		Non-blockers	
Organ toxicity	Hepatotoxicity	Inactive	Probability 0.69
	Neurotoxicity	Inactive	0.89

	Nephrotoxicity	active	0.62
	Respiratory toxicity	active	0.83
	Cardiotoxicity	Inactive	0.99
Toxicity end points	Carcinogenicity	Active	0.68
	Immunotoxicity	Inactive	0.97
	Mutagenicity	Active	0.51
	Cytotoxicity	Inactive	0.99
	BBB-barrier	Active	0.53
	Ecotoxicity	Inactive	0.53
	Clinical toxicity	Inactive	0.53
Molecular Initiating Events	Thyroid hormone receptor alpha (THR α)	Inactive	0.9
	Thyroid hormone receptor beta (THR β)	Inactive	0.78
	Transthyretin (TTR)	Inactive	0.97
	Ryanodine receptor (RYR)	Inactive	0.98
	GABA receptor (GABAR)	Inactive	0.96
	Glutamate N-methyl-D-aspartate receptor (NMDAR)	Inactive	0.92
	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPA)	Inactive	0.97
	Kainate receptor (KAR)	Inactive	0.99
	Achetylcholinesterase (AChE)	Inactive	0.69
	Constitutive androstane receptor (CAR)	Inactive	0.98
	Pregnane X receptor (PXR)	Inactive	0.92
	NADH-quinone oxidoreductase (NADHox)	Inactive	0.97
	Voltage gated sodium channel (VGSC)	Inactive	0.95
	Na ⁺ /I ⁻ symporter (NIS)	Inactive	0.98

Screened Luteolin Targets and Autism Targets

The SwissTargetPrediction database was employed to predict potential targets of luteolin, resulting in a total of 100 predicted targets. Additionally, 16,529 autism-related targets were retrieved from the GeneCards database, from which 4,067 genes with a reference score greater than 1 were selected for further analysis. By comparing luteolin targets with autism-related genes using a Venn diagram, 39 overlapping genes were identified as potential targets for luteolin

against autism, as shown in Figure 2. The corresponding Entrez IDs of these targets are provided in Table 2.

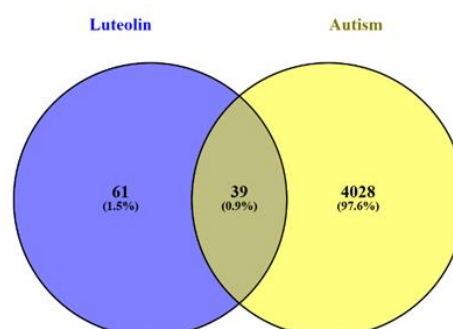


Figure 2 Venn diagram of Luteolin and autism related targets

Table 3 The detailed entrez IDs of 39 potential targets of Luteolin Against Autism

S.no	Symbol	Target	Entrez ID
1	<i>TERT</i>	Telomerase reverse transcriptase	O14746
2	<i>TTR</i>	Transthyretin	P02766
3	<i>ESR1</i>	Estrogen receptor	P03372
4	<i>APP</i>	Amyloid-beta precursor protein	P05067
5	<i>ARG1</i>	Arginase-1	P05089
6	<i>IGF1R</i>	Insulin-like growth factor 1 receptor	P08069
7	<i>ABCB1</i>	ATP-dependent translocase ABCB1	P08183
8	<i>MET</i>	Hepatocyte growth factor receptor (HGF receptor)	P08581
9	<i>PARP1</i>	Poly [ADP-ribose] polymerase 1	P09874
10	<i>AMY1A</i>	Alpha-amylase 1A	P0DUB6
11	<i>AR</i>	Androgen receptor	P10275
12	<i>TOP1</i>	DNA topoisomerase 1	P11387
13	<i>CYP19A1</i>	Aromatase	P11511
14	<i>SRC</i>	Proto-oncogene tyrosine-protein kinase Src	P12931
15	<i>CFTR</i>	Cystic fibrosis transmembrane conductance regulator	P13569
16	<i>TYR</i>	Tyrosinase	P14679
17	<i>MMP9</i>	Matrix metalloproteinase-9 (MMP-9)	P14780
18	<i>MAOA</i>	Amine oxidase [flavin-containing] A	P21397
19	<i>DRD4</i>	D(4) dopamine receptor	P21917
20	<i>ACHE</i>	Acetylcholinesterase (AChE)	P22303
21	<i>CA4</i>	Carbonic anhydrase 4	P22748
22	<i>CA6</i>	Carbonic anhydrase 6	P23280
23	<i>PIK3R1</i>	Phosphatidylinositol 3-kinase regulatory subunit alpha	P27986
24	<i>CD38</i>	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1	P28907
25	<i>ADORA2A</i>	Adenosine receptor A2a	P29274
26	<i>AVPR2</i>	Vasopressin V2 receptor (V2R)	P30518
27	<i>AKT1</i>	RAC-alpha serine/threonine-protein kinase	P31749
28	<i>ABCC1</i>	Multidrug resistance-associated protein 1	P33527
29	<i>PTGS2</i>	Prostaglandin G/H synthase 2	P35354
30	<i>AHR</i>	Aryl hydrocarbon receptor	P35869
31	<i>XDH</i>	Xanthine dehydrogenase/oxidase	P47989
32	<i>GSK3B</i>	Glycogen synthase kinase-3 beta	P49841
33	<i>BACE1</i>	Beta-secretase 1	P56817
34	<i>CSNK2A1</i>	Casein kinase II subunit alpha	P68400
36	<i>GLO1</i>	Lactoylglutathione lyase	Q04760
36	<i>PTK2</i>	Focal adhesion kinase 1 (FADK 1)	Q05397
37	<i>PTPRS</i>	Receptor-type tyrosine-protein phosphatase S (R-PTP-S)	Q13332
38	<i>CAMK2B</i>	Calcium/calmodulin-dependent protein kinase type II subunit beta	Q13554
39	<i>ESR2</i>	Estrogen receptor beta (ER-beta)	Q92731

PPI Network Analysis

A bar chart of degree value, highlighting the top 25 core targets, is shown in Figure 3a. These include *AKT1*, *SRC*, *ESR1*, *GSK3B*, *PTGS2*, *MMP9*, *IGF1R*, *PARP1*, *AR*, *ESR2*, *MET*, *ABCB1*, *APP*, *MAOA*, *TERT*, *PIK3R1*, *PTK2*, *ACHE*, *CYP19A1*, *CSNK2A1*, *TYR*, *XDH*, *BACE1*, *ADORA2A*, *ARG1*. A total of 39 predicted targets were imported into STRING for PPI network analysis (Figure 3.b). The network

complex included 39 nodes and 175 edges. The Cytoscape software was used to visualize and analyse the network by calculating centrality and other parameters. All the targets were arranged into circles according to these parameters Figure 3c. The high centrality value represented an important role in the network. Then, the CytoHubba selected the core targets (Figure 3d). The top 10 core targets were *AKT1*, *SRC*, *ESR1*, *GSK3B*, *PTGS2*, *MMP9*, *PARP1*, *IGF1R*, *AR*, *ESR2*, respectively.

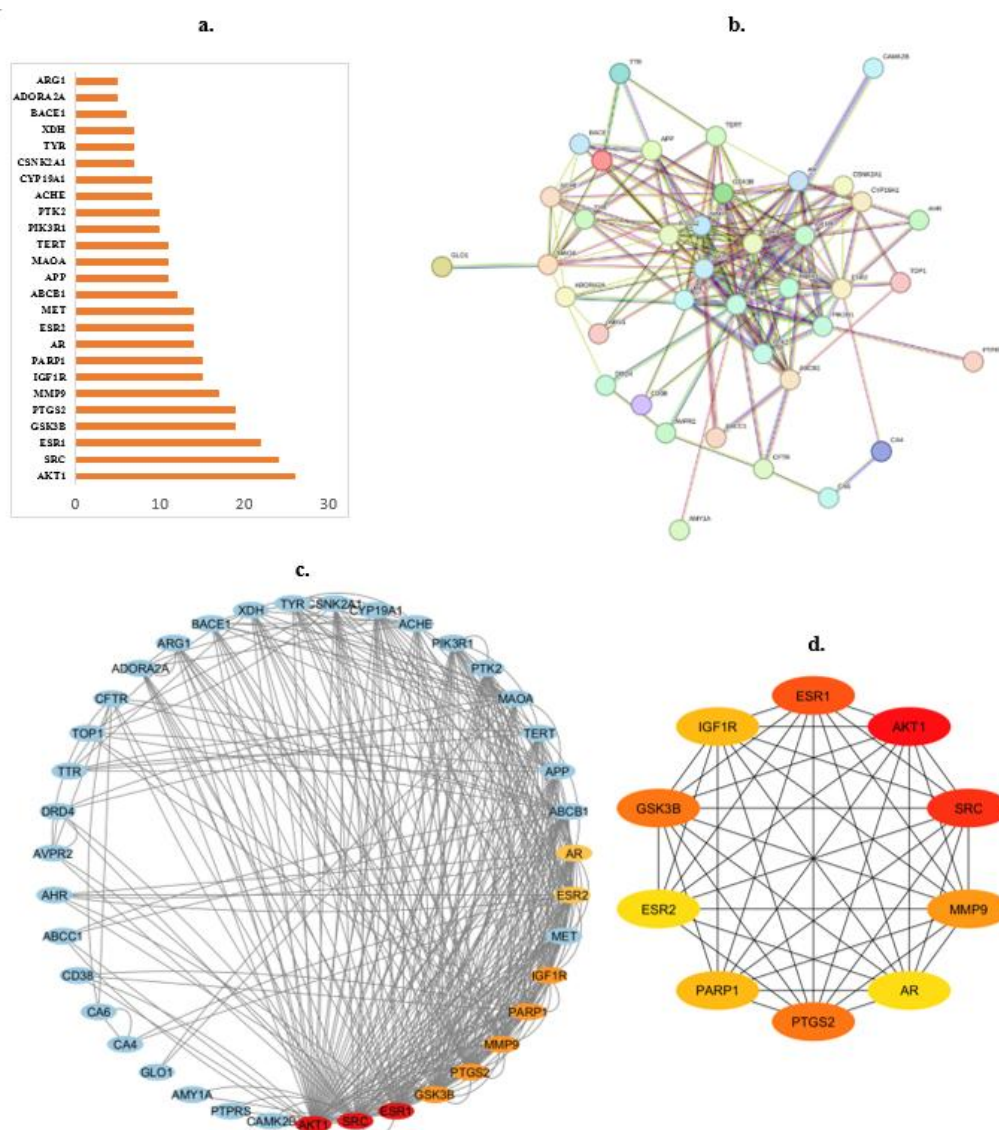


Figure 3a. Top 25 targets ranked by the degree value; b. PPI network of 39 common targets as per the STRING database; c. Molecular complexes; d. The top 10 core targets are visualized through the Cytoscape software.

GO enrichment analyses.

To determine the biological mechanisms of Luteolin Against Autism, we performed GO enrichment analysis on the 39 common targets between Luteolin and Autism using ShinyGO database. The analysis was divided into three categories: BP, CC, and MF and the top 15 GO terms were visualized in the bubble plot (Figure 4). In the GO enrichment bubble plot, bubble size represents the number of genes associated with each GO term, and bubble color corresponds to statistical significance ($-\log_{10}$ FDR). Our analysis revealed that the main enriched BP categories were the cellular response to hormone stimulus, Cellular response to oxygen-containing compound, Response to oxygen-containing compound, Reg. of programmed cell death, Reg. of cell population proliferation, Pos. reg. of cell communication, Pos. reg. of signaling, Pos. reg. of response to stimulus, Cell death, Cellular response to chemical stimulus, Reg. of signaling, Reg. of cell communication, Reg. of biological quality, Reg. of signal transduction, and Reg. of response to stimulus. The most strongly enriched CCs were the protein kinase complex and the serine/threonine protein kinase complex, transferring phosphorus-containing groups, Postsynapse, and Synapse. The most enriched MFs were Nitric oxide synthase regulator activity, Carbonate dehydratase activity, Estrogen response element binding, Transcription coactivator binding, Hydrolyase activity, Calmodulin binding, Lyase activity, Protein serine kinase activity, Protein kinase activity, Phosphotransferase activity (alcohol group as acceptor), Transferase activity transferring phosphorus-containing groups, Kinase activity, Zinc ion binding, Transition metal ion binding, and Identical protein binding.

prominently enriched, while post-synapse and synapse showed moderate enrichment with relevant significance levels. The main enriched MFs were Nitric-oxide synthase regulator activity, carbonate dehydratase activity, Estrogen response element binding, and transcription coactivator binding. Moderately enriched functions included hydrolyase activity, calmodulin binding, protein serine kinase activity, lyase activity, and protein kinase activity. Phosphotransferase activity (alcohol group as acceptor), transferase activity transferring phosphorus-containing groups, transition metal ion binding, zinc ion binding, and identical protein binding, showed smaller fold enrichments. Collectively, the enrichment analysis indicates that the gene set is strongly linked to regulatory pathways prevailing cellular signalling, stress responses, and cell survival, with a prominent association with kinase-containing protein complexes and related enzymatic activities. In addition, the results highlight molecules involved in protein-protein and protein-DNA interactions, along with a secondary enrichment in synaptic structural components.

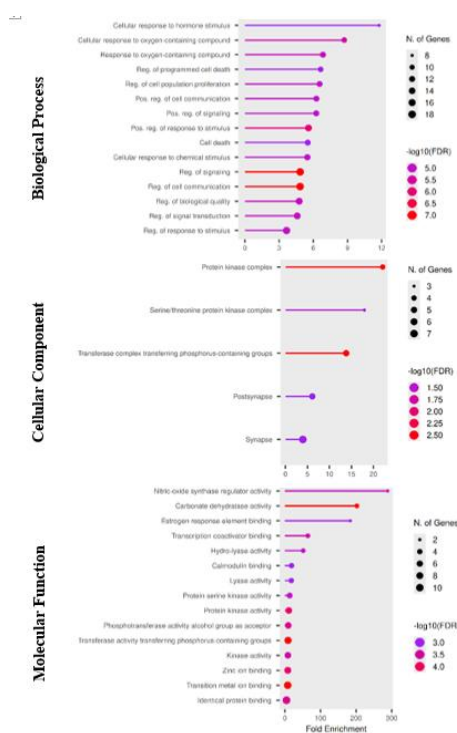


Figure 4- GO enrichment analyses of identified common targets using ShinyGo . a. Bar plot of top 15 biological processes, b. Bar plot of top 15 cellular components, c. Bar plot of top 15 molecular functions

KEGG pathway enrichment analyses

The metabolic pathways of potential therapeutic targets for the treatment of Autism were identified through KEGG pathway enrichment analyses. Using the ShinyGO database, we obtained top 20 signaling in a bar graph (Figure 5a). The results showed that the key targets were enriched in nitrogen metabolism, proximal tubule bicarbonate reclamation, and multiple intracellular signaling cascades. Several pathways under cancer categories, EGFR tyrosine kinase inhibitor resistance, endocrine resistance, dopaminergic synapse, HIF-1 signaling, pathways in cancer, hormone signaling, and PI3K-Akt signaling were also found. Pathways with direct relevance to neurodevelopment and autism, such as nitrogen metabolism, dopaminergic synapse pathway, PI3K-Akt signaling pathway, HIF-1 signaling, hormone and adrenal junction pathways relevant to autism biology are shown in Figure 5b.

In each pathway diagram, the detected genes are marked in red. Nitrogen Metabolism pathway map highlights carbonic anhydrase genes *CA2*, *CA4*, *CA5A*. Adherens Junction highlighted nodes corresponding to *CSNK2A*, *IGF1R*, and *MET*, located at key points within the cell-cell adhesion and signaling components of the pathway in Dopaminergic Synapse Four genes *DRD4*, *AKT1*,

MAOA, and *CAMK2B* appear highlighted within regions representing presynaptic signaling, neurotransmitter degradation, and postsynaptic signaling modules. HIF-1 Signaling Pathway figure contains highlighted positions for *AKT1*, *IGF1R*, and *CAMK2B*, mapped to signaling cascades involving HIF-1 regulation. PI3K-Akt Signaling Pathway Four genes *CDK6*, *AKT1*, *IGF1R*, and *MET* are marked in red within core components of the PI3K-Akt signaling cascade. These pathways were also chosen for molecular docking to validate the therapeutic effect of Luteolin Against Autism

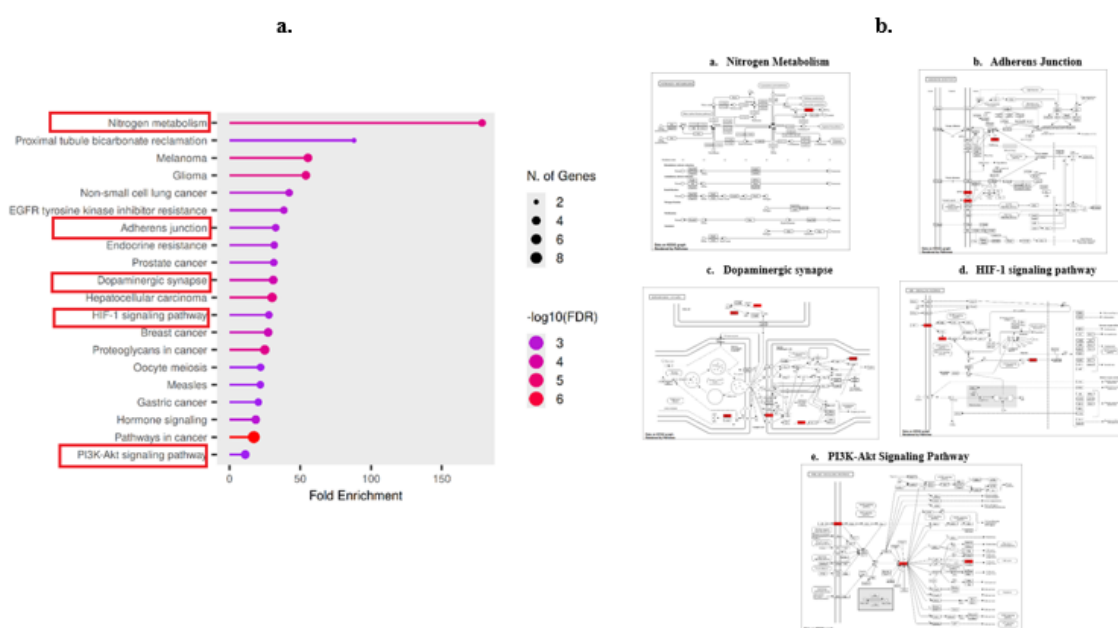


Figure 5 a. - Top 20 KEGG pathway analyses of identified common targets using Shinge, highlighting the most relevant pathways to autism biology; **b.** KEGG pathway maps showing the most relevant autism-associated pathways. a. Nitrogen metabolism Highlighted genes *CA2*, *CA4*, and *CA5A*; b. Adherens junction Highlighted genes *CSNK2A*, *IGF1R*, and *MET*. c. Dopaminergic synapse Highlighted genes *DRD4*, *AKT1*, *MAOA*, and *CAMK2B*; d. HIF-1 signalling pathway Highlighted genes *AKT1*, *IGF1R*, and *CAMK2B*; e. PI3K-Akt signalling pathway Highlighted genes *CDK6*, *AKT1*, *IGF1R*, and *MET*

Molecular docking analysis

Target proteins selected from signalling pathways, including CA4 (Carbonic anhydrase IV), MET (Hepatocyte Growth Factor Receptor), DRD4 (Dopamine D4 receptor), AKT1 (RAC- α serine/threonine kinase), and IGF1R (Insulin-like Growth Factor 1 receptor) were subjected to molecular docking analysis. Among these, AKT1 appeared as a common hub protein across three pathways. In comparison, MET and IGF1R were shared between two pathways, highlighting their central role in signaling. The 3D structure of Luteolin was retrieved from Pubchem in SDF format. Crystal structures of the selected proteins (PDB IDs: 1ZNC, 1RIW, 5WIU, 3O96, and 3LVP) were downloaded from the Protein Data Bank. Docking poses were visualized using protein cartoon and secondary-structure representations to evaluate cavity orientation, intermolecular interactions, and binding energies. All target-ligand complexes exhibited binding affinities lower than -7.0 kcal/mol, indicating a strong and favorable interaction between Luteolin and the selected receptor proteins, summarized in Table 4. Figure 6 illustrates the docked conformations and interaction networks with the highest relevance and docking scores. Luteolin formed a stable

interaction within the active pocket of Carbonic Anhydrase IV (CA4; PDB: 1ZNC) with a binding energy of -7.8 kcal/mol mainly through hydrogen bonding and hydrophobic contacts around T255 and K39. HGF receptor MET (PDB: 1RIW) exhibited a binding affinity of -7.7 kcal/mol, mainly with residues K1215 and N1178. Dopamine D4 receptor (DRD4; PDB: 5WIU), showing a binding energy of -8.9 kcal/mol and multiple stabilizing interactions, including hydrophobic contacts around V430 and polar bonds with R186. A notably high affinity was recorded for AKT1 (PDB: 3O96) with a docking score of -9.8 kcal/mol, mainly with residues A1505, K268, Q79. Luteolin bound to IGF1R (PDB: 3LVP) with -8.3 kcal/mol, forming interactions with residues M1111, Y1090, F1147. These docking results support the potential multi-target therapeutic mechanism of luteolin with multiple molecular targets like nitrogen metabolism, neuronal signaling, and PI3K-Akt-associated survival pathways

Table 4. Different binding energies of Luteolin with selected targets.

Pathway	Genes	Target name	PDB ID	Affinity (kcal/mol)
Nitrogen metabolism	CA4	Carbonic anhydrase IV	1ZNC	-7.8
Adherens junction	MET	Hepatocyte growth factor receptor (HGFR)	1RIW	-7.7
Dopaminergic synapse	DRD4	Dopamine D4 receptor	5WIU	-8.9
HIF-1 signaling pathway	AKT1	RAC-alpha serine/threonine-protein kinase	3O96	-9.8
PI3K-Akt signaling pathway	IGF1R	Insulin-like growth factor 1 receptor	3LVP	-8.3

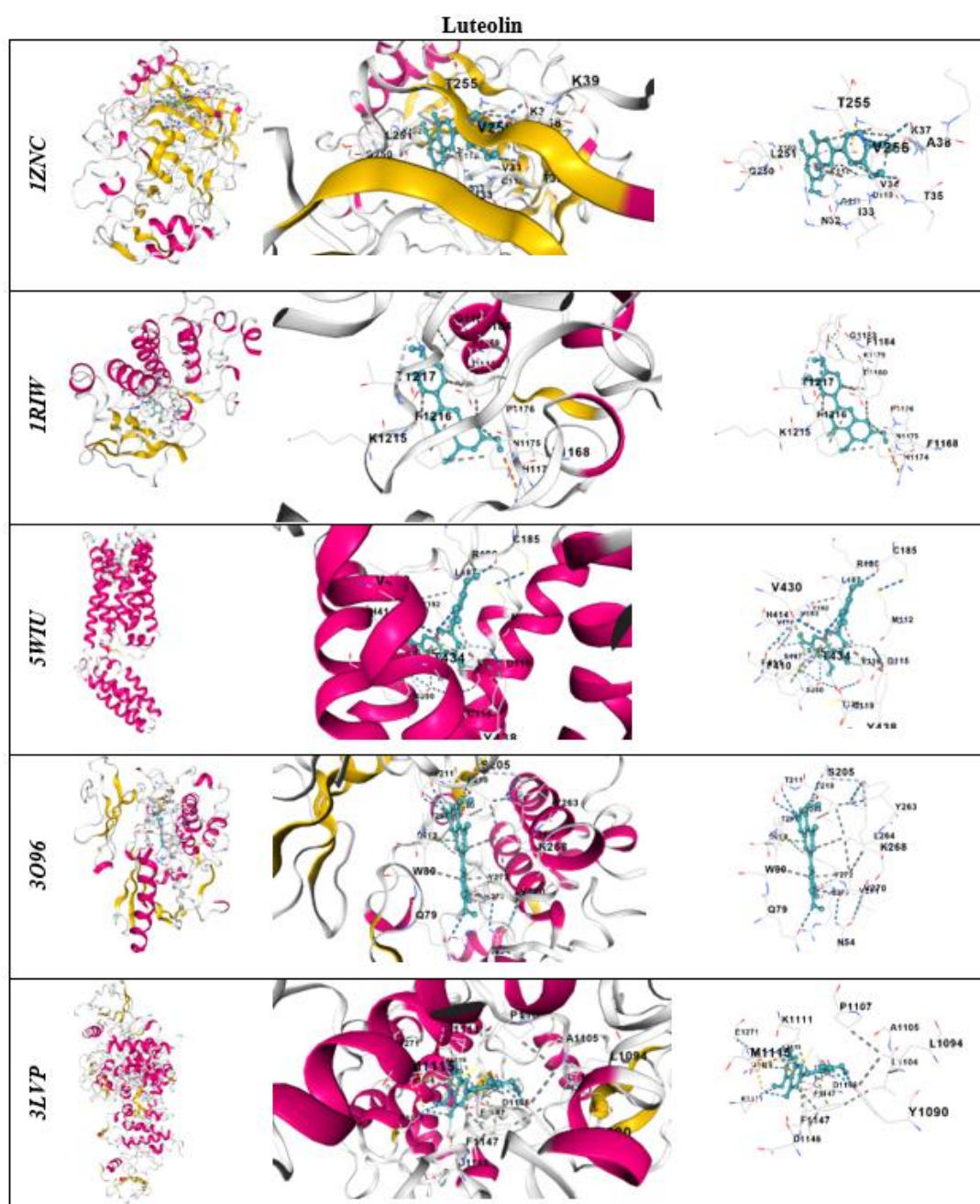


Figure 6. Molecular docking interaction profiles of luteolin with key target proteins identified from pathway enrichment analysis. *Docking poses for CA4 (1ZNC), MET (1RIW), DRD4 (5WIU), AKT1 (3O96), and IGF1R (3LVP) are shown along with their binding energies. 3D structures of protein with overall Docking with the Ligand, followed by Docking Complex and interaction map of the Ligand with Key Amino Acid*

Discussion

Preclinical studies and animal models have demonstrated that luteolin, a naturally occurring flavonoid with anti-inflammatory and antioxidant properties, can alleviate a number of symptoms associated with autism spectrum disorder (ASD). It is not a cure, though, and more thorough clinical trials are needed to prove its safety and effectiveness. This work elucidates how luteolin, a dietary flavonoid, might rectify many signaling pathways known to be impacted in ASD.

Luteolin demonstrated ASD-related gene binding activities. Among the potential targets for luteolin, nitrogen metabolism (*CA2*, *CA4*, *CA5A*), adherence junction (*CSNK2A*, *IGF1R*, *MET*); Dopaminergic signalling (*DRD4*, *AKT1*, *MAOA*, *CAMK2B*), HIF-1 signalling pathway (*AKT1*, *IGF1R*, *CAMK2B*) and PI3K-Akt signalling pathway (*CDK6*, *AKT1*, *IGF1R*, *MET*) were shown. Up to our understanding of the neurochemical etiological mechanisms of ASD, these could be easily related to luteolin protective and therapeutic effects of ASD.

In terms of nitrogen metabolism-related genes (*CA2*, *CA4*, *CA5A*), carbonic anhydrases enzymes may be linked to glutamate excitotoxicity, a major cause of autism. *CA2*, *CA4*, and *CA5A* are involved in nitrogen metabolism and the glutamate-glutamine cycle, which is essential for avoiding glutamate excitotoxicity, oxidative stress, and neuroinflammation in ASD by maintaining correct pH and ion balance. Normal activity is important to maintain the body's acid-base homeostasis systems, which indirectly support very sensitive nitrogen metabolism pathways, particularly in the brain. Based on this luteolin effect could be partly explained on the basis that, the molecular mechanism of synaptic transmission, which involves the coupling of electrical and chemical signals, is necessary for the integration of learning and memory functions. Membrane potential is necessary for nerve cell transmission. The movement of key ions across the membrane is essential to generate membrane potential, which may regulate dendritic excitability. Research suggests there is higher carbonic anhydrase (CA) II in some individuals with autism, potentially linked to zinc deficiency and heavy metal accumulation and brain development. Thus, luteolin can exert its therapeutic effects through the inhibition of carbonic anhydrases (CAs) and is regarded as a powerful inhibitor of human mitochondrial Cas. This inhibitory activity is due to the phenolic structure, which binds to the enzyme's active site. This is supported by a bioinformatic Indonesian investigation, which identified carbonic anhydrase II as a possible target protein. The survey results revealed that the

protein carbonic anhydrase II, a potential candidate for the causation of autism, can bind to heavy metals [19]. This can find support through the nitrogen metabolism pathway figure showing glutamine-glutamic cycle as part of the signalosome affected with luteolin. It is well accepted that mutations in the neuroligin (NLGN) and neurexin (NRXN) complexes, which encode cell adhesion molecules, have a key role in synaptic development, transcription, and excitatory-inhibitory balance. Synaptic disruption may play a role in the development of ASD [20]. Regarding adherence junction genes variants related to luteolin in the current study, *CSNK2A*, *IGF1R*, and *MET*, it is interesting to highlight that all been linked to an increased risk ASD, mostly through disruptions in brain development and synapse function.

Intestinal permeability is the sum of the functionally distinct tight junction pores and leaky routes. The tight junction pore route is a high capacity, size- and charge-selective conduit whose permeability is primarily regulated by a subset of zonulin and claudin family proteins [21]. Interestingly, inhibiting casein kinase 2 (*CSNK2A*) limits claudin-2 channel activity by preventing IL-13-induced claudin-2 overexpression, which increases gut permeability in vivo [22]. Furthermore, Dörfel *et al* [22] found that *CSNK2A* -dependent occludin phosphorylation reduced its binding to zonulin-2 protein, compromising tight junction integrity and increased intestinal permeability. Al-Ayaadhi *et al* [23] proved an interesting relationship between *CSNK2A* downregulation and glutamate excitotoxicity, neuro-inflammation, and leaky gut, as etiological mechanisms in ASD. Thus, luteolin could exert its therapeutic effects through the inhibition of *CSNK2A* which in turn can ameliorate increased intestinal permeability and glutamate excitotoxicity as important phenotypes of ASD directly related to ASD severity. This can find support in a study of Lolli *et al* [24] recording inhibition of *CSNK2A* by luteolin.

Insulin and insulin-like growth factor 1 (IGF1R) signaling are critical for maintaining brain homeostasis and controlling neurodevelopmental processes [25]. Insulin is involved in two key signaling cascades, AKT/mTOR and RAS/ERK, that control cellular growth, metabolism, and survival. Disruptions in these pathways have been significantly linked to a variety of neurodevelopmental diseases, most notably autism [26,27]. IGF-1, a neurotrophic factor required for healthy CNS development, regulates neuronal growth, synaptogenesis, survival, and migration [26]. It operates through endocrine, paracrine, and autocrine pathways via the widely expressed receptor IGF1R [26]. Systemic IGF1 can cross both the blood-brain barrier and the blood-cerebrospinal fluid barrier, impacting on early

CNS development and neuronal plasticity. Once bound to the IGF1 receptor, IGF1 activates the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) and phosphatidylinositol-3 kinase/mammalian target of rapamycin/serine-threonine-specific protein kinase (AKT-PKB PI3K/AKT1/mTOR) pathways, which are implicated in the processes of brain cell proliferation, neurogenesis, myelination, maturation, and differentiation and prevent apoptosis [27]. In fact, IGF1 enhances the secretion of various neurotransmitters and mediates the effects of other neurotrophic factors (i.e., BDNF and VEGF) [28,29].

In relation to dopaminergic signaling, research reveals a link between dopamine D4 receptor (DRD4) gene variations and autism spectrum disorder (ASD), notably in terms of repetitive and oppositional behaviors. The 7-repeat allele of the DRD4 gene has been associated to these problematic behaviors in children with ASD, although DRD4's overall significance in the etiology of autism is thought to be doubtful, with conflicting findings and additional research needed for confirmation [30]. Interestingly, ASD individuals with DRD4 gene polymorphism of heterozygous alleles with 7 repetitions are associated with a higher frequency of epilepsy as a co-morbidity related to oxidative stress, neuroinflammation, and glutamate excitotoxicity. The discovery of a higher frequency of epilepsy in individuals with DRD4 polymorphisms with 7 repeats raises questions about the involvement of the dopaminergic system in epileptogenesis, both in individuals with and without ASD [31]. Moreover, Qin et al [32] reported dopamine D4 receptor variant D4.7 induces over-suppression of NMDA glutamate receptor. As glutamatergic drugs, riluzole, a voltage-dependent sodium channel inhibitor and NMDA/kainate receptor antagonist, and memantine, a non-selective NMDA receptor antagonist, were found to ameliorate ASD symptoms in both children and adults [33,34].

This could help to support the presence of DRD4 among the recorded luteolin targets, as luteolin was recently reported to treat glutamate excitotoxicity as etiological mechanism of multiple neurological disorders among which is ASD [35]. This could be supported through considering the presence of AMPA, NMDA, kainite receptors with the related signaling molecules within the dopaminergic signaling presented in Figure 5b. Thus, the luteolin effect may be related to the refinement of glutamatergic signaling in ASD. This suggestion could find support in numerous in vitro and in vivo investigations, the natural flavonoid luteolin has shown notable neuroprotective benefits against glutamate excitotoxicity. It reduces inflammation, cell

death, and brain damage brought on by high glutamate levels through a variety of ways [36,37].

Calcium signaling regulates a variety of pathways critical for neuronal communication, synaptic plasticity, and information processing in the brain [38]. CAMK2B encodes a protein kinase that is essential for synaptic plasticity, learning, and memory, and its failure can alter brain development and function, resulting in a variety of neurological and behavioral disorders among which is ASD [39-41]. It has been reported that genetic variations that impact effector proteins of CaMK4 such as CREB and HDAC4 (histone deacetylase 4) also contribute to autism pathology. Furthermore, FRM1, a target gene of CREB, is strongly associated with autism. A crucial regulatory mechanism that actively balances excitatory and inhibitory impulses inside neurons to prevent the formation of hyperactivity or hypoactivity is homeostatic neuronal plasticity, which is largely maintained by the CAMK signaling pathway [42,43]. Thus, the presence of CAMK2B gene with the downstream signaling molecules could be easily linked to impaired plasticity, imbalanced inhibitory/excitatory phenomenon, neuroinflammation, and oxidative stress in ASD.

Previous research has identified CAMK2B as a modulator of endoplasmic reticulum (ER) stress, oxidative stress, and mitochondrial dysfunction, all are among the recorded etiological mechanisms of ASD, with CAMK2B inhibition emerging as a possible therapeutic pathway for treating several disorders. This could find support in the recent work of Umsumarn et al [44] which proved that luteolin exhibited anti-inflammatory properties in THP-1 cells by significantly suppressing IL-6, IL-8, and IL-1 β cytokine secretion in a dose-dependent manner. Furthermore, they reported that luteolin exhibited the downregulation of the MAPK pathway, as evidenced by modulating the phosphorylation of p-ERK1/2, p-JNK and p-p38 proteins.

The presence of HIF signalling pathway genes and enzymes among the luteolin targets detected by networking could be supported by the fact that increased serum HIF-1 α and apelin levels may have a role in the etiopathogenesis of ASD [45]. Hypoxia signaling has been associated to autism spectrum disorder (ASD), especially in the context of prenatal and perinatal hypoxia, which can raise ASD risk. A shortage of oxygen activates the hypoxia-inducible factor-1 α (HIF-1 α) protein, which plays a vital role in the cellular response to stress. In the case of ASD, this signaling can result in undesirable effects such as decreased brain development, disrupted synaptic plasticity, and neuroinflammation [45].

The PI3K-Akt signaling system is involved in autism spectrum disorder (ASD) because it regulates cell formation, survival, and protein synthesis, and its disruption is connected to ASD pathogenesis. Upregulation of the PI3K-AKT/mTOR signalling pathway involves many human brain abnormalities, including autism and other neurological dysfunctions. In contrast, other studies suggest that individuals with autism have decreased Akt phosphorylation, which may be associated with other biochemical abnormalities such as lower GABA levels. Therefore, inhibition of the PI3K-AKT/mTOR signalling pathway may have an effective therapeutic value for autism treatment. Post-mortem examinations of people with autism spectrum disorder (ASD) found an increased density of excitatory synapses in their brains, which could be attributable to abnormal mTOR-dependent synaptic pruning. Furthermore, in silico modelling in TSC2 haploinsufficient mice revealed mTOR-dependent increased spine density associated with ASD-like stereotypes and corticostriatal hyperconnectivity. However, injection of the mTORC1 inhibitor rapamycin abolished these effects [46]

The recorded targets' relevance as therapeutic signaling associated to the therapeutic efficacy of luteolin, an important dietary flavonoid with antioxidant and anti-inflammatory characteristics, may assist to emphasize the importance of these targets in the etiopathology of autism. To analyze molecular docking between luteolin and proteins (Figure 6 and Table 4), we consider the binding affinity or docking score, binding pose or particular orientation, and interactions, such as hydrogen bonds and hydrophobic interactions, within the protein's active region. A higher docking score or more negative binding energies indicate a greater affinity, whereas the binding pose shows the exact chemical interactions that stabilize the complex. Luteolin docking with targets revealed great affinity and stabilized interactions.

This could help to conclude that: Luteolin can be quite helpful in the treatment of ASD, and this study can help us to highlight the numerous targets and signaling involved. One of the most important signaling pathways that could be targeted is unbalanced excitatory/inhibitory neurotransmission or glutamate excitotoxicity, which has been identified as an etiological mechanism in autism spectrum disorder. Unintentionally, the four chosen pathways representing nitrogen metabolism, adherence junction, HIF-signaling, and PI3K-Akt signaling were directly or indirectly associated with glutamate excitotoxicity, which has recently been reported as an etiology that can be

reserved to improve the clinical presentation of autism spectrum disorder [47]

Theoretical Validation of Findings

The multi-target profile predicted for luteolin aligns with established etiological mechanisms of ASD, including glutamate excitotoxicity, oxidative stress, neuroinflammation, synaptic dysfunction, and altered growth factor signaling [48, 49]. The enrichment of pathways such as PI3K-Akt, HIF-1, dopaminergic synapse, and adherens junction signaling has been independently reported in ASD literature, supporting the plausibility of our computational results. The identification of AKT1, MET, IGF1R, DRD4, and CAMK2B as central nodes is consistent with prior research implicating these proteins in neuronal development, synaptic plasticity, mitochondrial dysfunction, and neuroimmune imbalance [50-52]. Thus, the network-based predictions are strongly corroborated by previously documented ASD molecular signatures, providing theoretical validation for our proposed mechanisms.

Limitations

This study is based entirely on in silico analyses, including network pharmacology, enrichment analysis, and molecular docking. Although these computational predictions provide strong theoretical insight into luteolin's multitarget mechanisms against ASD, they cannot fully substitute for experimental evidence. Future laboratory validation is therefore essential to confirm the predicted interactions and signaling pathways.

Recommendations for Future Work

Experimental validation is required to confirm luteolin's predicted interactions with key ASD-related targets and pathways, including AKT1, MET, IGF1R, DRD4, CAMK2B, PI3K-Akt, and HIF-1. In vitro neuronal assays, in vivo ASD models, glutamate-GABA analyses, and carbonic anhydrase activity studies will be essential to verify its effects on excitotoxicity, inflammation, and neurodevelopment. These investigations will strengthen the translational relevance of the computational results.

List of Abbreviations

ASD	Autism Spectrum Disorder
PPI	Protein-Protein Interaction
GO	Gene Ontology

KEGG	Kyoto Encyclopedia of Genes and Genomes
CAMK2B	Calcium/Calmodulin-Dependent Protein Kinase Type II Beta
AKT1	RAC-alpha Serine/Threonine-Protein Kinase
IGF1R	Insulin-Like Growth Factor 1 Receptor
DRD4	Dopamine D4 Receptor
CA	Carbonic Anhydrase
MAPK	Mitogen-Activated Protein Kinase
ADMET	Absorption, Distribution, Metabolism, Excretion & Toxicity
BBB	Blood-Brain Barrier
PDB	Protein Data Bank
BP/CC/MF	GO Biological Process / Cellular Component / Molecular Function
HIF-1	Hypoxia-Inducible Factor-1
PI3K-Akt	Phosphatidylinositol-3-Kinase-Akt Pathway

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References

- Mottron, L.; Bzdok, D. Autism spectrum heterogeneity: Fact or artifact? *Mol. Psychiatry* 2020, 25, 3178–3185.
- Zeidan, J.; Fombonne, E.; Scorah, J.; Ibrahim, A.; Durkin, M.S.; Saxena, S.; Yusuf, A.; Shih, A.; Elsabbagh, M. Global prevalence of autism: A systematic review update. *Autism Res.* 2022, 15, 778–790.
- Sauer AK, Stanton JE, Hans S, et al. Autism Spectrum Disorders: Etiology and Pathology. In: Grabrucker AM, editor. *Autism Spectrum Disorders* [Internet]. Brisbane (AU): Exon Publications; 2021 Aug 20. Chapter 1. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK573613/> doi: 10.36255/exonpublications.autismspectrumdisorders.2021.etiology
- Cai JL, Lu JQ, Lu G, "Autism spectrum disorder related tcm symptoms and tcm herbs prescriptions: a systematic review and meta-analysis," *North American Journal of Medicine and Science*, vol. 8, no. 1, 2015.
- Bang M, Lee SH, Cho SH et al., "Herbal medicine treatment for children with autism spectrum disorder: a systematic review," *Evidence-based Complementary and Alternative Medicine: eCAM*, vol. 2017, Article ID 8614680, 2017.
- Zhang, R, X. Zhu, H. Bai, and K. Ning, "Network pharmacology databases for traditional Chinese medicine: review and assessment," *Frontiers in Pharmacology*, vol. 10, p. 123, 2019.
- Harwood M, nielewska-Nikiel B, Borzelleca JF, Flamm GW, Williams GM, Lines TC. A critical review of the data related to the safety of quercetin and lack of evidence of in vivo toxicity, including lack of genotoxic/carcinogenic properties. *Food Chem Toxicol* 2007; 45: 2179–2205.
- Kawanishi S, Oikawa S, Murata M. Evaluation for safety of antioxidant chemopreventive agents. *Antioxid Redox Signal* 2005; 7: 1728–1739. [DOI] [PubMed] [Google Scholar]
- Li L, Gu L, Chen Z, Wang R, Ye J, Jiang H. Toxicity study of ethanolic extract of *Chrysanthemum morifolium* in rats. *J Food Sci* 2010; 75: T105–T109.
- Jager AK, Saaby L. Flavonoids and the CNS. *Molecules* 2011; 16: 1471–1485.
- Savino, R.; Medoro, A.; Ali, S.; Scapagnini, G.; Maes, M.; Davinelli, S. The Emerging Role of Flavonoids in Autism Spectrum Disorder: A Systematic Review. *J. Clin. Med.* 2023, 12, 3520. <https://doi.org/10.3390/jcm12103520>
- T. Ling, W. Wang, C. Hu et al., "[Effects of orchinol on invasion, migration and Wnt3a/β-catenin signaling pathway of human gastric cancer SGC-7901 cells]," *Zhongguo Zhongyao Zazhi*, vol. 43, no. 23, pp. 4718–4723, 2018.
- M. B. Alam, N. S. Chowdhury, M. H. Sohrab, M. S. Rana, C. M. Hasan, and S.-H. Lee, "Cerevisterol alleviates inflammation via suppression of MAPK/NF-κB/AP-1 and activation of the Nrf2/HO-1 signaling cascade," *Biomolecules*, vol. 10, no. 2, p. 199, 2020.
- P. Srikantha and M. H. Mohajeri, "The possible role of the microbiota-gut-brain-Axis in autism spectrum disorder," *International Journal of Molecular Sciences*, vol. 20, no. 9, p. 2115, 2019.
- K. Sowndhararajan, P. Deepa, M. Kim, S. J. Park, and S. Kim, "An overview of neuroprotective and cognitive enhancement properties of lignans from *Schisandra chinensis*," *Biomedicine & Pharmacotherapy*, vol. 97, pp. 958–968, 2018.
- Khlood Mohammed Mehdar, Saad Misfer Alqahtani. Protective Effects of Luteolin on a Rat Model of Autism: An Analysis of Luteolin Flavonoid's Effects on Rat Behaviour, Histology and Cerebellar Pathology. *Int. J. Pharmacol.* 2024, 20(6), 942–955. <https://doi.org/10.3923/ijp.2024.942.955>
- Tsilioni I, Taliou A, Francis K, Theoharides TC. Children with autism spectrum disorders, who improved with a luteolin-containing dietary formulation, show reduced serum levels of TNF and IL-6. *Transl Psychiatry.* 2015 Sep 29;5(9):e647. doi: 10.1038/tp.2015.142.
- El-Ansary, A.K., Bacha, A.B. & Kotb, M. Etiology of autistic features: the persisting neurotoxic effects of propionic acid. *J Neuroinflammation* 9, 74 (2012). <https://doi.org/10.1186/1742-2094-9-74>
- Dian Eka A. F. Ningrum¹, Mohamad Amin, Betty Lukiat. *Bioinformatics Approach Based Research of*

- Profile Protein Carbonic Anhydrase II Analysis as a Potential Candidate Cause Autism for The Variation of Learning Subjects Biotechnology. JURNAL PENDIDIKAN BIOLOGI INDONESIA VOLUME 3 NOMOR 1 TAHUN 2017 (Halaman 28-35)
20. Sindi IA. Implications of Cell Adhesion Molecules in Autism Spectrum Disorder Pathogenesis. *J Microsc Ultrastruct.* 2022 Aug 4;11(4):199-205. doi: 10.4103/jmau.jmau_15_22.
 21. Shen L., Weber C.R., Raleigh D.R., Yu D., Turner J.R. Tight Junction Pore and Leak Pathways: A Dynamic Duo. *Annu. Rev. Physiol.* 2011;73:283-309. doi: 10.1146/annurev-physiol-012110-142150.
 22. Dörfel M.J., Westphal J.K., Bellmann C., Krug S.M., Cording J., Mittag S., Tauber R., Fromm M., Blasig I.E., Huber O. CK2-dependent phosphorylation of occludin regulates the interaction with ZO-proteins and tight junction integrity. *Cell Commun. Signal.* 2013;11:40. doi: 10.1186/1478-811X-11-40.
 23. Al-Ayadhi L, Bhat RS, Alghamdi FA, Alhadlaq AS, El-Ansary A. Influence of Auditory Integrative Training on Casein Kinase 2 and Its Impact on Behavioral and Social Interaction in Children with Autism Spectrum Disorder. *Curr Issues Mol Biol.* 2023 May 15;45(5):4317-4330. doi: 10.3390/cimb45050274.
 24. Lolli, Graziano & Cozza, Giorgio & Mazzorana, Marco & Tibaldi, Elena & Cesaro, Luca & Donella-Deana, Arianna & Meggio, Flavio & Venerando, Andrea & Franchin, Cinzia & Sarno, Stefania & Battistutta, Roberto & Pinna, Lorenzo. (2012). Inhibition of Protein Kinase CK2 by Flavonoids and Tyrphostins. *A Structural Insight. Biochemistry.* 51. 6097-107. 10.1021/bi300531c.
 25. Vasic, V.; Jones, M.S.O.; Haslinger, D.; Knaus, L.S.; Schmeisser, M.J.; Novarino, G.; Chiocchetti, A.G. Translating the Role of mTOR- and RAS-Associated Signalopathies in Autism Spectrum Disorder: Models, Mechanisms and Treatment. *Genes* 2021, 12, 1746.
 26. Réthelyi, J.M.; Vincze, K.; Schall, D.; Glennon, J.; Berkel, S. The role of insulin/IGF1 signalling in neurodevelopmental and neuropsychiatric disorders—Evidence from human neuronal cell models. *Neurosci. Biobehav. Rev.* 2023, 153, 105330.
 27. Robinson-Agramonte, M.L.A.; Michalski, B.; Vidal-Martinez, B.; Hernández, L.R.; Santiesteban, M.W.; Fahnestock, M. BDNF, proBDNF and IGF-1 serum levels in naïve and medicated subjects with autism. *Sci. Rep.* 2022, 12, 13768.
 28. Riikonen, R. Insulin-Like Growth Factors in the Pathogenesis of Neurological Diseases in Children. *Int. J. Mol. Sci.* 2017, 18, 2056. [Google Scholar] [CrossRef]
 29. Netchine, I.; Azzi, S.; Le Bouc, Y.; Savage, M.O. IGF1 molecular anomalies demonstrate its critical role in fetal, postnatal growth and brain development. *Best practice research. Clin. Endocrinol. Metab.* 2011, 25, 181-190.
 30. Haider Mohsen Kazem, Hadeel Rashid Faraj, Tariq Khudair Hussein .Genetic Analysis of Dopamine Receptors (DRD4 and DRD5) in Patients with Autism with Attention Deficit Hyperactivity Disorder (ADHD) in Thi-Qar Governorate -Iraq *Journal of Bioscience and Applied Research*, 2025, Vol. 11, No. 3, P.892-901 pISSN: 2356-9174, eISSN: 2356-9182
 31. Wehmuth M, Antoniuk SA, Da Silva KB, Raskinb S, Oliveira Christoff AD, et al. (2020) Dopamine DRD4 gene polymorphism as a risk factor for epilepsy in autism spectrum disorder. *J Biol Med* 4(1): 012-017. DOI: <https://dx.doi.org/10.17352/jbm.000021>
 32. Qin L, Liu W, Ma K, Wei J, Zhong P, Cho K, Yan Z. The ADHD-linked human dopamine D4 receptor variant D4.7 induces over-suppression of NMDA receptor function in prefrontal cortex. *Neurobiol Dis.* 2016 Nov;95:194-203. doi: 10.1016/j.nbd.2016.07.024. Epub 2016 Jul 27
 33. Shamabadi, Ahmad, Hanie Karimi, Razman Arabzadeh Bahri, Mohsen Motavaselian, and Shahin Akhondzadeh. "Emerging drugs for the treatment of irritability associated with autism spectrum disorder." *Expert Opinion on Emerging Drugs* 29, no. 1 (2024): 45-56.
 34. Doyle, C. A., & McDougale, C. J. (2012). Pharmacotherapy to control behavioral symptoms in children with autism. *Expert Opinion on Pharmacotherapy*, 13(11), 1615-1629.
 35. Wu, Wl., Gong, Xx., Qin, Zh. et al. Molecular mechanisms of excitotoxicity and their relevance to the pathogenesis of neurodegenerative diseases—an update. *Acta Pharmacol Sin* 46, 3129-3142 (2025). <https://doi.org/10.1038/s41401-025-01576-w>
 36. Tzu Yu Lin, Cheng Wei Lu, Su Jane Wang, Luteolin protects the hippocampus against neuron impairments induced by kainic acid in rats, *Neuro Toxicology*, Volume 55, 2016, Pages 48-57, <https://doi.org/10.1016/j.neuro.2016.05.008>.
 37. Vongthip W, Nilkhet S, Boonruang K, Sukprasansap M, Tencomnao T, Baek SJ. Neuroprotective mechanisms of luteolin in glutamate-induced oxidative stress and autophagy-mediated neuronal cell death. *Sci Rep.* 2024 Apr 2;14(1):7707. doi: 10.1038/s41598-024-57824-2. PMID: 38565590; PMCID: PMC10987666.
 38. Kawamoto EM, Vivar C, Camandola S (2012): Physiology and pathology of calcium signaling in the brain. *Front Pharmacol* 3:61.
 39. Akita, T., Aoto, K., Kato, M., Shiina, M., Mutoh, H., Nakashima, M., ... & Saitsu, H. (2018). De novo variants in CAMK 2A and CAMK 2B cause neurodevelopmental disorders. *Annals of clinical and translational neurology*, 5(3), 280-296.
 40. Küry, S., van Woerden, G. M., Besnard, T., Onori, M. P., Latypova, X., Towne, M. C., ... & Mercier, S. (2017). De novo mutations in protein kinase genes CAMK2A and CAMK2B cause intellectual disability. *The American Journal of Human Genetics*, 101(5), 768-788.
 41. Dwyer Bonnie K. , Veenma Danielle C. M. , Chang Kiki , Schulman Howard , Van Woerden Geeske M. Case Report: Developmental Delay and Acute Neuropsychiatric Episodes Associated With a de novo Mutation in the CAMK2B Gene (c.328G>A p.Glu110Lys) *Frontiers in Pharmacology* Volume 13 - 2022, DOI=10.3389/fphar.2022.794008

42. Nardone S, Sams DS, Reuveni E, Getselter D, Oron O, Karpuj M, Elliott E (2014): DNA methylation analysis of the autistic brain reveals multiple dysregulated biological pathways. *Transl Psychiatry* 4:e433.
43. Jacqueline Kaiser, Alana Risteska, Abbey G. Muller, Haoxiong Sun, Bethany Lei, Kevin Nay, Anthony R. Means, Margot A. Cousin, David H. Drewry, Jonathan S. Oakhill, Bruce E. Kemp, Anthony J. Hannan, Michael Berk, Mark A. Febbraio, Andrew L. Gundlach, Elisa L. Hill-Yardin, John W. Scott, Convergence on CaMK4: A Key Modulator of Autism-Associated Signaling Pathways in Neurons, *Biological Psychiatry*, Volume 97, Issue 5, 2025, Pages 439-449, <https://doi.org/10.1016/j.biopsych.2024.10.012>.
44. Umsumarnng, S.; Dissook, S.; Arjsri, P.; Srisawad, K.; Thippraphan, P.; Sangphukieo, A.; Thongkumkoon, P.; Dejkriengkraikul, P. Inhibitory Effect of Luteolin on Spike S1 Glycoprotein-Induced Inflammation in THP-1 Cells via the ER StressInducing Calcium/CHOP/MAPK Pathway. *Pharmaceuticals* 2024, 17, 1402. <https://doi.org/10.3390/ph17101402>
45. Esen Yildirim Demirdöğen, Mehmet Akif Akinci, Abdullah Bozkurt, Özgür Esmeray, Fatma Betül Özgeriş, Nezahat Kurt, Neslihan Yüce, Serum hypoxia-inducible factor-1 alpha (HIF-1 α) and apelin levels in children and adolescents diagnosed with autism spectrum disorder, *Research in Autism Spectrum Disorders*, Volume 112, 2024, 102327, <https://doi.org/10.1016/j.rasd.2024.102327>.
46. Pagani, M., Barsotti, N., Bertero, A., Trakoshis, S., Ulysse, L., Locarno, A., Miseviciute, I., De Felice, A., Canella, C., Supekar, K., Galbusera, A., Menon, V., Tonini, R., Deco, G., Lombardo, M. V., Pasqualetti, M., & Gozzi, A. (2021). mTOR-related synaptic pathology causes autism spectrum disorder-associated functional hyperconnectivity. *Nature communications*, 12(1), 6084. <https://doi.org/10.1038/s41467-021-26131-z>
47. El-Ansary A, GABA and glutamate imbalance in autism and their reversal as novel hypothesis for effective treatment strategy 2020 *Autism Dev. Disord* 18 3 46-63
48. Vongthip W, Nilkhet S, Boonruang K, Sukprasansap M, Tencomnao T, Baek SJ. Neuroprotective mechanisms of luteolin in glutamate-induced oxidative stress and autophagy-mediated neuronal cell death. *Sci Rep*. 2024 Apr 2;14(1):7707. doi: 10.1038/s41598-024-57824-2. PMID: 38565590; PMCID: PMC10987666.
49. Grabarczyk, M.; Justyńska, W.; Czapkowska, J.; Smolińska, E.; Bielenin, A.; Glabinski, A.; Szpakowski, P. Role of Plant Phytochemicals: Resveratrol, Curcumin, Luteolin and Quercetin in Demyelination, Neurodegeneration, and Epilepsy. *Antioxidants* 2024, 13, 1364. <https://doi.org/10.3390/antiox13111364>
50. Enriquez-Barreto L, Morales M. The PI3K signaling pathway as a pharmacological target in Autism related disorders and Schizophrenia. *Mol Cell Ther*. 2016 Feb 11;4:2. doi: 10.1186/s40591-016-0047-9. PMID: 26877878; PMCID: PMC4751644.
51. Kosillo P, Bateup HS. Dopaminergic Dysregulation in Syndromic Autism Spectrum Disorders: Insights From Genetic Mouse Models. *Front Neural Circuits*. 2021 Jul 23;15:700968. doi: 10.3389/fncir.2021.700968. PMID: 34366796; PMCID: PMC8343025.
52. Baranova J, Dragunas G, Botelho MCS, Ayub ALP, Bueno-Alves R, Alencar RR, Papaiz DD, Sogayar MC, Ulrich H, Correa RG. Autism Spectrum Disorder: Signaling Pathways and Prospective Therapeutic Targets. *Cell Mol Neurobiol*. 2021 May;41(4):619-649. doi: 10.1007/s10571-020-00882-7. Epub 2020 May 28. PMID: 32468442; PMCID: PMC11448616.