



Establishing network pharmacology between natural polyphenols and Alzheimer's disease using bioinformatic tools – An advancement in Alzheimer's research

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ABSTRACT

Alzheimer's disease (AD) is a major cause of disability and one of the top causes of mortality globally. AD remains a major public health challenge due to its prevalence, impact on patients and caregivers, and the current lack of a cure. In recent years, polyphenols have garnered attention for their potential therapeutic effects on AD. The objective of the study was to establish network pharmacology between selected polyphenols of plant origin and AD. Insilico tools such as SwissADME, ProTox3.0, pkCSM, Swiss Target Prediction, DisGeNET, Inter-ActiVenn, DAVID database, STRING database, Cytoscape/CytoHubba were employed to establish the multi-target potential of the polyphenolic compounds. The present study revealed that out of 17 polyphenols, 10 ligands were found to possess a drug-likeness nature along with desirable pharmacokinetic parameters and a lesser toxicity profile. Also, the results highlighted the possible interactions between the polyphenols and the disease targets involved in AD. Further, this study has shed light on the mTOR pathway and its impact on AD through the autophagic mechanism. Overall, this study indicated that polyphenols could be a better therapeutic option for treating AD. Hence, the consumption of polyphenolic cocktails as a part of the diet could produce more effective outcomes against the disease. Additional studies are warranted in the future to explore additional pathways and genes to provide a comprehensive understanding regarding the usage of the shortlisted polyphenols and their derivatives for the prevention and treatment of AD.

1. Introduction

Alzheimer's disease (AD) primarily targets the brain leading to progressive cognitive decline and memory loss. The disease is characterized by the accumulation of abnormal protein aggregates, especially beta-amyloid plaques and tau tangles, that disrupt neuronal function and communication [1,2]. The limited effectiveness of current pharmaceuticals has prompted the exploration of alternative therapies. One promising avenue is the use of natural polyphenols, abundant in fruits, vegetables, and plants, known for their potential neuroprotective effects against cognitive decline and AD pathogenesis [3]. Recent advances in bioinformatics and network pharmacology have enabled systematic

exploration of the anti-AD properties of these compounds, establishing the complex interactions and therapeutic potentials of natural polyphenols against AD.

The last decade has witnessed accelerating interest in the prevention and treatment of neurodegenerative diseases, particularly AD, through natural polyphenols. This interest is rooted in the ability of polyphenols to modulate various biological pathways implicated in the onset and progression of AD [4]. Studies have highlighted how dietary polyphenols can exert neuroprotective [5] and pro-cognitive activities by modulating oxidative stress and inflammatory responses, which are cardinal features in AD pathophysiology [6]. Preclinical evidence supports the potential of polyphenols like resveratrol, curcumin, ellagic

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acid, quercetin, etc., in counteracting AD's pathogenic processes through their modulation of oxidative stress and inflammation [7].

Oxidative stress plays a pivotal role in AD's pathogenesis, serving as a target for therapeutic strategies. Natural polyphenolic compounds have been noted for their antioxidant neuroprotective effects and therapeutic potential for AD [8]. Also, compounds such as curcumin, epigallocatechin-3-gallate (EGCG), resveratrol, and tannic acid, have shown promising effects in decreasing amyloid-beta (A β) production, preventing or altering A β aggregation, and reducing oligomer cytotoxicity [9]. These natural molecules highlight the broad spectrum of polyphenols in offering potential AD therapeutic strategies.

The multifaceted actions of polyphenols offer significant therapeutic potential, addressing various pathological aspects of AD from amyloid-beta aggregation to oxidative stress and neuroinflammation [10]. Thus, natural polyphenols not only hold therapeutic promise but also inspire new research directions for developing novel AD treatments. Polyphenols are compounds with a wide spectrum of complex structures prevalent in diets based on plants. Polyphenols can be classified as either phenolic alcohols or phenolic acids. Based on the strength of their phenolic ring, polyphenols may be divided into many different forms, but the main groups include phenolic acids, flavonoids, stilbenes, phenolic alcohols, and lignans. Numerous edible and medicinal plants, as well as fruits, vegetables, tea, red wine, and extra virgin olive oil, are significant food sources that contain polyphenols [11]. These phytochemicals are known to shield human health from long-term degenerative diseases. They exhibit several protective properties against cancer, neurological disorders, atherosclerosis, stroke, and cardiovascular diseases [12,13].

The integration of network pharmacology into allopathic medicine research has inaugurated a new era of drug discovery and development [14], characterized by a deeper understanding of drug actions at both the molecular and system levels [15]. This approach propels the study of drugs from a new perspective, emphasizing a holistic view over the reductionist methods traditionally employed in pharmaceutical research. It assesses the interplay between drugs and disease targets within the vast network of biological pathways, inherently relying on a synergistic approach to treatment.

2. Materials and method

2.1. Search strategy

Natural polyphenols have always been seen to exhibit beneficial effects on various diseases. Based on a literature survey that was carried out using various search engines such as Scopus, Proquest, PubMed, and Google Scholar, utilizing the keywords, 'Alzheimers', 'polyphenols' 'neuroprotection', 'anti-aging', and 'AD' [16], the following compounds including Chlorogenic Acid, Ferulic acid, Ellagic Acid, Genistein, Curcumin, Kaempferol, Lignan, Luteolin, Resveratrol, Pterostilbene, Naringenin, Quercetin, Rottlerin, Berberine, Rutin, Silymarin, and Apigenin were reflected as search results. These 17 compounds (refer to the structures of the ligands in the supplementary file) have been observed to be associated with neuroprotection and anti-aging effects and are beneficial against neurodegenerative diseases including Alzheimer's disease [17–33].

2.2. Screening of compounds

2.2.1. Drug-likeness prediction

The canonical smiles of the selected 17 polyphenols (depicted in Table 1) were obtained from the PubChem database and entered into the SwissADME web server (<http://www.swissadme.ch/>) to assess their drug-likeness. Several parameters were used to assess the drug-like properties of the polyphenols, including lead-likeness and bioavailability score, TPSA (Topological Polar Surface Area), which ascertain the brain permeability and the gastrointestinal absorption of the

Table 1

List of compounds along with their canonical smiles used in our study.

S.No	Compound Name	Canonical smiles
1.	Chlorogenic Acid	<chem>C1C(C(C(C(C1(=O)O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O</chem>
2.	Ellagic Acid	<chem>C1=C2C3=C(C(=C1O)O)OC(=O)C4=CC(=C(C(=C43)OC2=O)O)O</chem>
3.	Curcumin	<chem>COC1=C(C=CC(=C1)C=CC(=O)CC(=O)C=CC2=CC(=C(C(=C2)O)OC)O</chem>
4.	Ferulic acid	<chem>COC1=C(C=CC(=C1)C=CC(=O)O)O</chem>
5.	Kaempferol	<chem>C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C(=C3O2)O)O)O)O</chem>
6.	Genistein	<chem>C1=CC(=CC=C1C2=COC3=CC(=CC(=C3C2=O)O)O)O</chem>
7.	Lignan	<chem>CCOC(=O)C1C(C(=O)C2=CC(=C(C=C2C1C3=CC(=C(C(=C3)OC)OC)OC)OC)O)C</chem>
8.	Luteolin	<chem>C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C(=C3O2)O)O)O)O</chem>
9.	Naringenin	<chem>C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC=C(C(=C3)O)O</chem>
10.	Quercetin	<chem>C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C(=C3O2)O)O)O)O)O</chem>
11.	Resveratrol	<chem>C1=CC(=CC=C1C=CC2=CC(=CC(=C2)O)O)O</chem>
12.	Rottlerin	<chem>CC1=C(C(=C(C(=C1O)C(=O)C)O)CC2=C(C(=C3C(=C2O)C=CC(O3)C)C)C(=O)C=CC4=CC=CC(=C4)O)O</chem>
13.	Rutin	<chem>CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC(=C(C(=C5)O)O)O)O)O)O)O</chem>
14.	Silymarin	<chem>COC1=C(C=CC(=C1)C2C(OC3=C(O2)C=C(C(=C3)C4C(C(=O)C5=C(C=C(C(=C5O4)O)O)OC)O</chem>
15.	Apigenin	<chem>C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C(=C3O2)O)O)O</chem>
16.	Berberine	<chem>COC1=C(C2=C[N+](=C(C=C2C=C1)C4=CC5=C(C=C4CC3)OCOC5)OC</chem>
17.	Pterostilbene	<chem>COC1=CC(=CC(=C1)C=CC2=CC=C(C(=C2)O)OC</chem>

selected ligands [34], iLOGP (lipophilicity, solubility in lipid), ESOL Log S and ESOL class (water solubility), and the Lipinski rule of five (molecular mass should be less than 500 Dalton, lipophilicity should be high, hydrogen bond donors should be less than five, hydrogen bond acceptors should be less than ten, and molar refractivity within (40–130) [35]. A bioavailability value of F > 30 % is required, where F30 indicates a 30 % bioavailability. Bioavailability is the pace and degree of absorption of the active ingredients from a drug product into the bloodstream.

With a Boiled Egg graphical interface, the drug-likeness is represented by ADME (Absorption, Distribution, Metabolism, and Excretion) characteristics, where the X and Y axes were displayed with TPSA and WLogP values, respectively. TPSA and WLogP stand for the ligand's accessibility and lipophilicity, respectively.

2.2.2. ADMET profiling

Utilizing the ProTox 3.0 web server (<https://tox.charite.de/protox3/#>), the toxicity profile of each of the 17 ligands was assessed [36]. The SMILES of the corresponding ligands were incorporated into the webserver to analyze and make an assumption of the compounds' LD₅₀ value. The compounds were categorized into Class 1–6 based on their toxicity nature. Class 1 indicates the highly toxic nature of the compound and Class 6 indicates the non-toxic nature of the compound. A limit of Class 4 was chosen as the main filter to eliminate some compounds out of 17 compounds used in our study [37]. Additionally, pkCSM (<https://biosig.unimelb.edu.au/pkcsm/>) webserver was employed to extensively assess the comprehensive pharmacokinetic profile of the ligands including parameters such as water solubility, Caco2 permeability, Intestinal absorption, Skin permeability, P-glycoprotein inhibition, volume of distribution, BBB permeability, CNS permeability, Enzyme interactions, and Renal clearance were assessed extensively to filter the potential compounds against AD [38,39].

2.2.3. Prediction of putative targets

Following screening via the SwissADME, ProTox-3.0 databases, and pkCSM web server, the canonical SMILES of the 10 chosen compounds were entered into the Swiss target prediction website and then analyzed to anticipate the possible targets.

2.3. Protein-protein interaction (PPI) network construction

The great diversity, flexibility, and selectivity of protein-protein interactions (PPI) make them extremely important [40]. The functional relationships between major targets were found using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) [41]. The CytoHubba plugin for Cytoscape was applied to the PPI network that was acquired from STRING to examine its core regulatory genes and identify relevant targets. Also, extended PPI network analysis was done to assess the clustering coefficient, number of nodes, number of edges, network radius, and average number of neighbours to validate the PPI [42].

2.4. Gene Ontology and KEGG pathway enrichment analysis

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway [43] enrichment and Gene Ontology (GO) enrichment were performed by employing the Database for Annotation, Visualization, and Integrated Discovery (DAVID) to identify the interactions between molecules and biological processes linked to specific hub genes.

2.5. Statistical analyses

Statistical techniques such as network centrality measures, and pathway analysis were performed to evaluate the significance of our findings.

3. Results

3.1. Screening of compounds

3.1.1. Drug-likeness prediction

Using Swiss ADME, the ligands were predicted for drug-likeness by utilizing the Ghosh filter and Lipinski's rule of five. For every ligand, drug-likeness properties such as logP, MW, number of hydrogen-bond donors (HBD), number of hydrogen-bond acceptors (HBA), number of hydrogen-bond donors (HBD), MLOGP, WLOGP, MR, and number of atoms were predicted. Out of 17 ligands, 13 compounds exhibited drug-likeness characteristics after passing the Lipinski and Ghose Filter with no violations. The compounds such as Chlorogenic acid, Rottlerin, Rutin, and Silymarin showed violations against Lipinski and Ghose filters. Fig. 1 depicts the findings for each ligand's drug-likeness.

The boiled egg graphical representation (displayed in Fig. 2), showed that Pterostilbene, Berberine, Resveratrol, and Ferulic acid have a good probability of crossing the BBB and the other polyphenols have a lower probability of crossing BBB, except Chlorogenic acid, Silymarin, and Rottlerin which were found to be non-brain penetrant [44,45].

3.1.2. ADMET profiling

Utilizing the ProTox 3.0 web server toxicity profile for all 17 polyphenols was assessed (depicted in Fig. 3). The results indicated that except for lignan, berberine, and quercetin, the remaining ligands showed high LD₅₀ values. The BBB permeability nature of the compounds predicted using ProTox - 3.0 was found to be inconsistent with the results evaluated through the SwissADME Boiled egg plot. To minimize this bias, the pkCSM web server was used to extensively study the comprehensive ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of the compounds

Generally, in the pkCSM web server, the value of water solubility ranges from -6 to 0. Lower values indicate poorer water solubility which may have challenges in absorption and formulation. The range of Caco2 permeability between -1 and 1.5 implies the ability of the compound to permeate the intestinal lining. A higher value suggests good permeability and potential for high oral bioavailability, while a lower value indicates poor absorption. The range of intestinal

Ligands	Molecular Formula	Molecular Weight (MW)	H-bond acceptor	H-bond donors	MLOGP	WLOGP	MR	No. of atoms	Bioavailability Score	Lipinski Filter (violation)	Ghose Filter (violation)
Chlorogenic Acid	C ₁₆ H ₁₈ O ₉	354.31	9	6	-1.05	-0.75	83.5	25	0.11	1	1
Ellagic Acid	C ₁₄ H ₆ O ₈	302.19	8	4	0.14	1.31	75.31	22	0.55	0	0
Curcumin	C ₂₁ H ₂₀ O ₆	368.38	6	2	1.47	3.15	102.8	27	0.55	0	0
Ferulic acid	C ₁₈ H ₁₆ O ₄	194.18	4	2	1	1.39	51.63	14	0.85	0	0
Kaempferol	C ₁₅ H ₁₀ O ₆	286.24	6	4	-0.03	2.28	76.01	21	0.55	0	0
Genistein	C ₁₅ H ₁₀ O ₅	270.24	5	3	0.52	2.58	73.99	20	0.55	0	0
Lignan	C ₂₅ H ₃₀ O ₈	458.5	8	0	1.58	3.87	121.76	33	0.55	0	0
Luteolin	C ₁₅ H ₁₀ O ₆	286.24	6	4	-0.03	2.28	76.01	21	0.55	0	0
Naringenin	C ₁₅ H ₁₂ O ₅	272.25	5	3	0.71	2.19	71.57	20	0.55	0	0
Quercetin	C ₁₅ H ₁₀ O ₇	302.24	7	5	-0.56	1.99	78.03	22	0.55	0	0
Resveratrol	C ₁₄ H ₁₂ O ₃	228.24	3	3	2.26	2.76	67.88	17	0.55	0	0
Rottlerin	C ₃₀ H ₂₈ O ₈	516.54	8	5	1.66	5.18	145.1	38	0.55	1	2
Rutin	C ₂₇ H ₃₀ O ₁₆	610.52	16	10	-3.89	-1.69	141.38	43	0.17	3	4
Silymarin	C ₂₂ H ₂₂ O ₁₀	482.44	10	5	-0.4	1.71	120.55	35	0.55	0	1
Apigenin	C ₁₅ H ₁₀ O ₅	270.24	5	3	0.52	2.58	73.99	20	0.55	0	0
Berberine	C ₂₀ H ₁₈ NO ₄ ⁺	336.36	4	0	2.19	3.1	94.87	25	0.55	0	0
Pterostilbene	C ₁₆ H ₁₆ O ₃	256.3	3	1	2.76	3.36	76.82	19	0.55	0	0

Fig. 1. Drug-likeness Prediction using the SwissADME database. Lipinski filter: MW≤500, MLOGP ≤4.15, N or O ≤10, NH or OH≤5; Ghosh filter:160≤MW≤480, -0.4≤WLOGP≤5.6, 40 ≤MR ≤130, 20 ≤atoms ≤70, F ≥ 30 %.

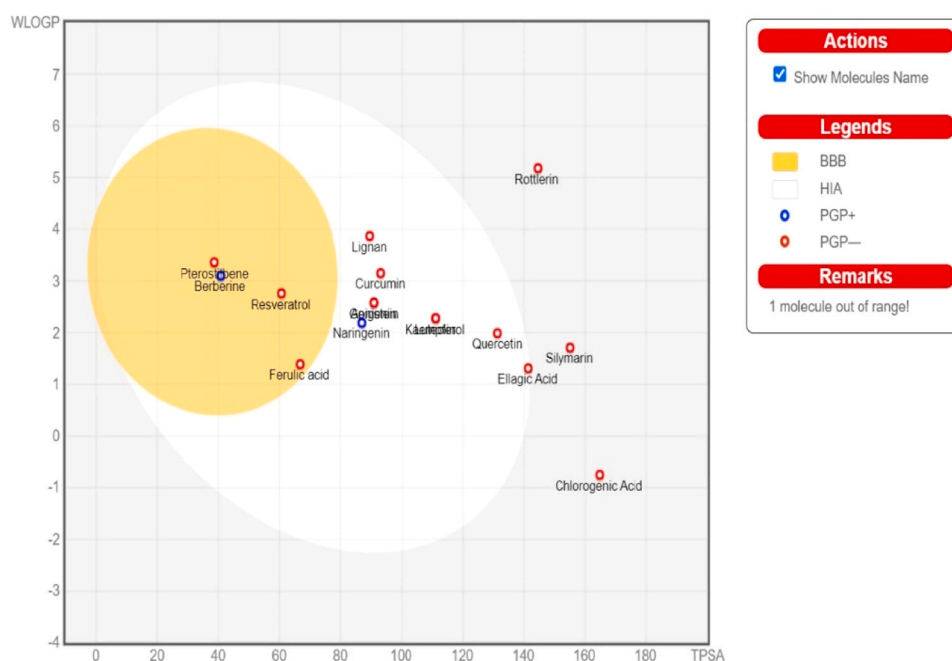


Fig. 2. Boiled egg graphical representation of 17 polyphenols using SwissADME.

Ligands	LD ₅₀ Value (mg/kg)	Toxicity Class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	Neurotoxicity	BBB
Chlorogenic Acid	5000	5	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active
Ellagic Acid	2991	4	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Active
Curcumin	2000	4	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active
Ferulic acid	1772	4	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active
Kaempferol	3919	5	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Active
Genistein	2500	5	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Active
Lignan	899	4	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active
Luteolin	3919	5	Inactive	Active	Inactive	Active	Inactive	Inactive	Active
Naringenin	2000	4	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Active
Quercetin	159	3	Inactive	Active	Inactive	Active	Inactive	Inactive	Active
Resveratrol	1560	4	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Rottlerin	1000	4	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active
Rutin	5000	5	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive
Silymarin	2000	4	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive
Apigenin	2500	5	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Berberine	200	3	Inactive	Active	Active	Active	Inactive	Active	Active
Pterostilbene	1560	4	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Active

Fig. 3. Toxicity results using ProTox – 3.0 Software. LD₅₀: Median Lethal Dose; BBB: Blood Brain Barrier.

absorption lies between 0 and 100. Compounds with values above 80 % are considered to have high absorption.

The skin permeability value ranges between -3 and -1 . Lower values suggest low skin permeability of the compounds indicating the compound's relevancy for topical or transdermal drug delivery. The P-glycoprotein inhibition property indicates the compound potentially affects the drug efflux and increases the intracellular drug

concentration. The volume of distribution value ranges between -2 and 2 . A higher value indicates the wide distribution of the compound throughout the tissues, and a lower value indicates the confinement of the compound to the plasma.

BBB permeability value ranges between -3 and 1 . Values closer to 0 or positive indicate good BBB permeability. Also, the CNS permeability ranges between -4 and 0 . Higher values indicate better CNS

penetration, which is very important for drugs targeting the brain. The enzyme interaction property indicates the possibility of the compound causing drug-drug interactions. Total clearance value ranges between −1 and 2. Higher values suggest faster clearance, which can affect dosing frequency and duration of action. The substrate property of compounds for Renal Organic cation transporter 2 implies the renal excretion via this transporter. If the compounds show no interaction, it indicates alternative pathways for renal excretion.

From the pkCSM webserver analysis (depicted in Fig. 4), it was found that 10 compounds including Ellagic acid, Ferulic acid, Kaempferol, Genistein, Luteolin, Naringenin, Quercetin, Apigenin, Resveratrol, and Pterostilbene exerted moderate to good oral bioavailability with variations depending on solubility and glycoside forms, their ability to cross BBB. Also, they have shown extensive phase II metabolism (especially glucuronidation and sulfation) and efficient renal clearance primarily through urine as conjugated metabolites. These properties play a crucial role in determining their potential therapeutic applications and dosage forms, so they were selected for further study, and 7 ligands such as Chlorogenic acid, Curcumin, Lignan, Rottlerin, Rutin, Silymarin, and Berberine with unfavourable pharmacokinetic profiles were removed from the further study.

3.1.3. Prediction of putative targets

Using the Swiss Target Prediction database, a total of 1000 target genes (refer to the supplementary file) were identified. After discarding the duplicated genes in the InterActVenn (<http://www.interactvenn.net/>), 343 putative target genes of 10 chosen active compounds were obtained (refer to the supplementary file). Following the identification of drugs’ promising targets, 3397 genes linked to AD were obtained from the DisGeNET database (refer to the supplementary file). Afterward, a Venn diagram was used to anticipate the shared targets of the compound-related genes and AD (shown in Fig. 5). A total of 203 putative genes that protect against AD were chosen and regarded as hub

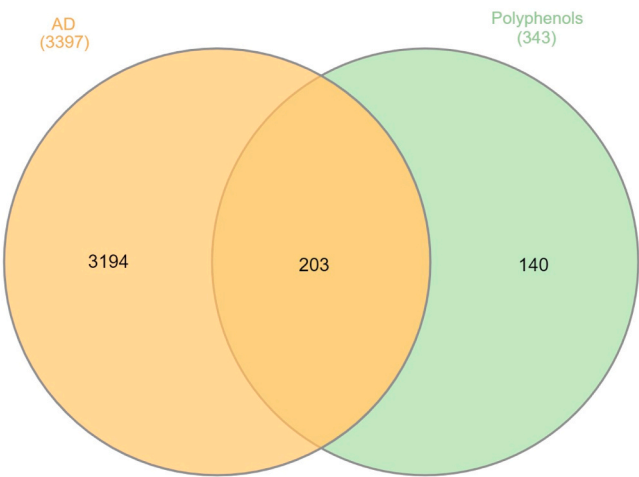


Fig. 5. Venn diagram showing the common genes between AD and the selected polyphenols.

targets.

3.2. Protein-protein interaction (PPI) network construction

One useful resource for predicting protein-protein interactions is the STRING (Search Tool for the Retrieval of Interacting Genes) database. The PPI network (displayed in Fig. 6) of gene lists was predicted using the STRING database version 12.0 (refer to the supplementary file). Cytoscape (v3.10.1) was used to show the predicted PPI network (shown in Fig. 7). Using Maximal Clique Centrality (MCC) topological analysis, the Cytoscape plugin CytoHubba (depicted in Fig. 8) was able to identify the top 10 core genes (BCL2, NFKB1, STAT3, ESR1, CTNNB1, MTOR,

Ligands	Water solubility (log mol/L)	Caco2 permeability (log Papp in 10 ⁻⁶ cm/s)	Intestinal absorption (human) (%) Absorbed)	Skin Permeability (log Kp)	P-glycoprotein I inhibitor	P-glycoprotein II inhibitor	VDss (human) (log L/kg)	BBB permeability (log BB)	CNS permeability (log PS)	CYP2D6 substrate	CYP3A4 substrate	CYP2D6 inhibitor	CYP3A4 inhibitor	Total clearance (log ml/min/kg)	Renal OCT2 substrate
Apigenin	-3.329	1.007	93.25	-2.735	No	No	0.822	-0.734	-2.061	No	No	No	No	0.566	No
Silymarin	-3.204	0.435	61.861	-2.735	Yes	Yes	0.369	-1.207	-3.639	No	No	No	No	-0.103	No
Rutin	-2.892	-0.949	23.446	-2.735	No	No	1.663	-1.899	-5.178	No	No	No	No	-0.369	No
Rottlerin	-2.972	-0.306	72.957	-2.735	Yes	Yes	-0.414	-1.472	-2.903	No	Yes	No	No	0.017	No
Resveratrol	-3.178	1.17	90.935	-2.737	No	No	0.296	-0.048	-2.067	No	Yes	No	No	0.076	No
Quercetin	-2.925	-0.229	77.207	-2.735	No	No	1.559	-1.098	-3.065	No	No	No	No	0.407	No
Naringenin	-3.224	1.029	91.31	-2.742	No	No	-0.015	-0.578	-2.215	No	No	No	No	0.06	No
Luteolin	-3.094	0.096	81.13	-2.735	No	No	1.153	-0.907	-2.251	No	No	No	No	0.495	No
Lignan	-5.67	1.283	100	-2.736	Yes	Yes	-0.73	-1.17	-3.273	No	Yes	No	Yes	0.341	No
Genistein	-3.595	0.9	93.387	-2.735	No	No	0.094	-0.71	-2.048	No	No	No	No	0.151	No
Kaempferol	-3.04	0.032	74.29	-2.735	No	No	1.274	-0.939	-2.228	No	No	No	No	0.477	No
Ferulic acid	-2.817	0.176	93.685	-2.72	No	No	-1.367	-0.239	-2.612	No	No	No	No	0.623	No
Curcumin	-4.01	-0.093	82.19	-2.764	Yes	Yes	-0.215	-0.562	-2.99	No	Yes	No	Yes	-0.002	No
Ellagic Acid	-3.181	0.335	86.684	-2.735	No	No	0.375	-1.272	-3.533	No	No	No	No	0.537	No
Chlorogenic Acid	-2.449	-0.84	36.377	-2.735	No	No	0.581	-1.407	-3.856	No	No	No	No	0.307	No
Pterostilbene	-3.905	1.729	92.395	-2.691	No	No	0.23	0.317	-1.635	No	Yes	No	No	0.228	No
Berberine	-3.973	1.734	97.147	-2.576	No	Yes	0.58	0.198	-1.543	No	Yes	Yes	Yes	1.27	No

Fig. 4. Comprehensive ADMET Profiling using pkCSM software. Caco2: Cancer coli-2 cells; VD_{ss}: The steady-state Volume of distribution; CNS permeability: Central Nervous System permeability; CYP2D6: Cytochrome P450 2D6; CYP3A4: Cytochrome P450 3A4; Renal OCT2: Renal Organic cation transporter 2.

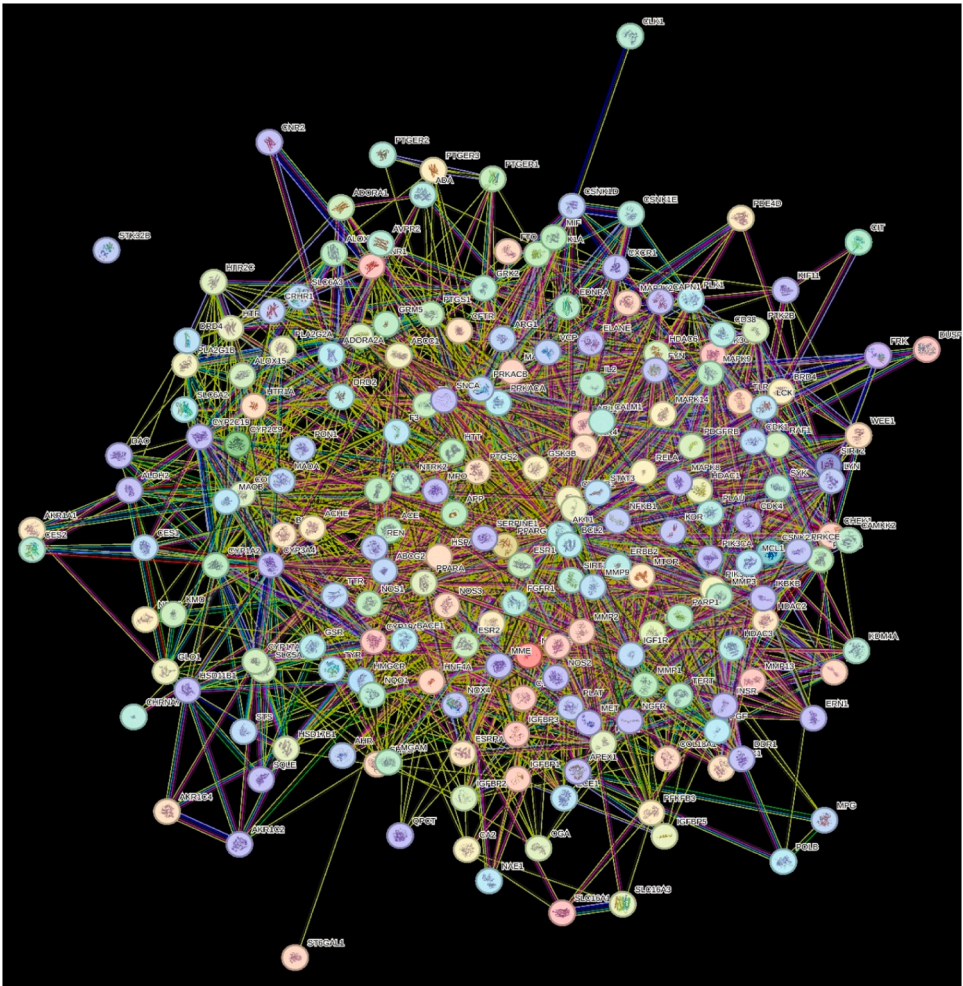


Fig. 6. PPI Network analysis between selected polyphenols and targets for AD obtained using STRING Database (12.0).

ACHE	PTGS2	HMGR	ALOX12	PTGS1	ADORA2A	MMP13	CTNNA1	MAPK8	AVPR2	ADORA1	COMT	PGF	TYR
HTR1A	FRK	MCL1	HDAC6	PTPN1	AKT1	HSD11B1	SLC16A3	ALOX5	CYP2C9	ACE	PTGER3	CAPN1	POLB
SERPINE1	MAPT	NOS1	CYP11A2	ELANE	CAMK2	CALM1	CYP2C19	SHBG	PTK2B	ALOX15	AKR1C2	KIF11	F3
CES2	FGFR1	CYP17A1	CES1	TLR4	STS	ABL1	PDE4D	PON1	PLG	SIRT2	ADA	ESR2	PRKACB
HDAC1	CYP19A1	PTGER1	NAE1	MME	NFKB1	CTSB	IL2	MAPK14	PIK3R1	DYRK1A	SLC5A2	PFKFB3	BCL2
HDAC3	COL18A1	ALDH2	CDK4	PLAT	LYN	IKBKB	PIK3CA	PLA2G2A	F2	FLT4	SQLE	DRD4	GLO1
NOX4	MTOR	MMP1	IGFBP1	FTO	CNR2	ABCC1	ECE1	AR	REN	DDR1	GRK2	CXCR1	SIRT1
BACE1	ERBB2	TERT	MAOA	STGAL1	CIT	GRM5	MMP3	HTR2A	DRD2	NQO2	CSNK2A1	SLC6A3	PPARG
CHRNA7	PLK1	ERN1	HDAC2	RXRA	CNR1	ABCB1	EDNRA	EGFR	HNFA4	PIK3CG	APP	BCHE	PTGER2
CYP3A4	MPG	GSR	CDK1	APEX1	HSPA1A	SLC6A2	TLR9	MIF	CFTR	CLK1	GSK3B	RAF1	PLAU
NOS3	MMP2	MAPK9	TTR	MMP9	NOS2	KDR	AHR	WEE1	KDM4A	HTR2C	ARG1	MPO	AKR1A1
QPCT	ESRRA	PLA2G1B	KMO	CSNK1E	HSD17B1	NQO1	DAPK1	NFE2L2	IGFBP5	MAP2K2	CHEK1	PARP1	PRKCE
ESR1	RELA	PDGFRB	MAOB	INSR	HTT	SLC16A1	DAO	ABCG2	NGFR	PIK3CB	STAT3	CD38	NTRK2
VCP	DUSP16	CSNK1D	PPARA	MGAM	CRHR1	OGA	IGFBP3	MET	FYN	PRKACA	LCK	CA2	BRD4
SNCA	IGFBP2	AKR1C4	IGF1R	SYK									

Fig. 7. PPI Network using Cytoscape (v3.10.1).

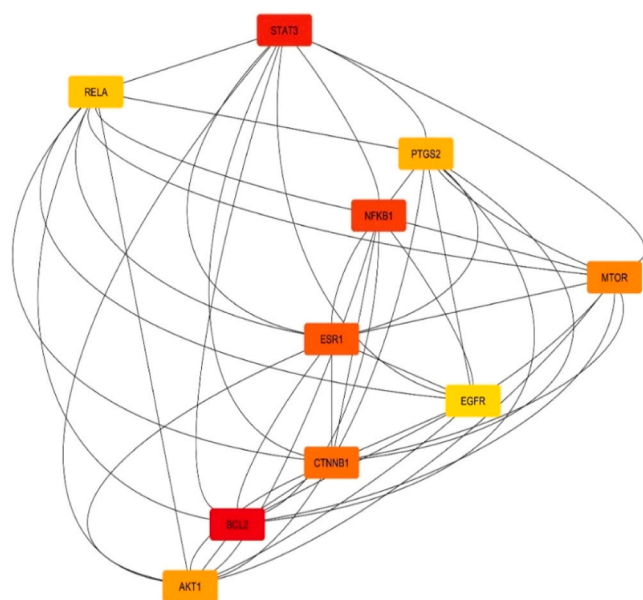


Fig. 8. Top 10 genes in Cytoscape (v3.10.1) sorted by MCC method. The node colour changes from red to yellow reflecting the rank from high to low in the network.

AKT1, PTGS2, RELA, EGFR). Also, detailed protein-protein interaction network analysis was performed and it indicated that there were 201 proteins (nodes) in the network (displayed in Fig. 9). About 2848 interactions were found between the proteins with an average of each protein interacting with approximately 28 other proteins with a network diameter of 7 steps and radius of 1 suggesting the network is highly connected. This network centrality measure showed significant validation of PPI performed in our study.

3.3. GO (Gene Ontology) enrichment analysis

Functional annotation and enrichment analysis were carried out using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) [46]. DAVID was used to estimate the function of the primary targets at three different levels: cellular component (CC), molecular function (MF), and biological process (BP). To further investigate the 203 selected target genes, GO enrichment analysis was executed on them (refer to the supplementary file). GO enrichment analysis revealed that most of the target genes were involved in biological processes (BP), cellular components (CC), and molecular functions (MF) (depicted in Fig. 10). Autophagy, Nuclear factor kappa B transduction, Amyloid beta

clearance, memory process, and regulation of ROS biosynthetic process in particular dominated the enriched BP ontologies. In the CC analysis, the amyloid and microsome account for the majority (203 target genes). Tyrosine-protein kinase, dioxygenase, peroxidase, and other enzymes dominated the enriched MF ontologies.

3.4. KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis

Following the GO enrichment analysis, KEGG pathway enrichment analysis of the 203 targeted genes was performed using the DAVID database (refer to the supplementary file). The results of the KEGG pathway enrichment analysis showed that 277 signal pathways and 203 putative target genes had significant associations ($FDR < 0.05$). In Fig. 11, the top 10 pathways with the highest enrichment ratios are shown.

According to the KEGG enrichment analysis of the top 10 pathways associated between the selected polyphenols and AD, the most relevant pathway is the mTOR Signaling Pathway involving the hub genes. Now previously using Maximal Clique Centrality (MCC) topological analysis, Cytoscape plugin CytoHubba we found the top 10 target genes involved between the disease and the drug [47]. By correlating these results, we can conclude that the mTOR signaling pathway can play a vital role in AD [48]. Therefore, this can be focused on treating AD using the selected polyphenols to get a more appropriate outcome.

4. Discussion

The study of natural polyphenols in the context of AD using bioinformatic tools presents a promising frontier in neurodegenerative disease research. Advancements in bioinformatics have facilitated the identification of targets, biomarkers, pathways, and potential therapeutics, integrating computational methods into the study of complex diseases like AD [14]. This approach enhances the understanding of AD's pathological mechanisms and the druggability of molecular targets, leveraging the vast potential of natural polyphenols systematically and efficiently.

This work serves as both a benchmark for the preliminary screening of some polyphenols and a novel therapeutic idea for more investigation into the processes underlying the use of polyphenols in the treatment of AD. After the initial scrutiny, 10 compounds with desirable pharmacokinetic parameters were further subjected to PPI and Enrichment analysis. The anti-AD targets of certain polyphenols were mostly related to the autophagy mechanism, memory, and regulation of the ROS biosynthetic process as per GO functional analysis. Also, the studies on the KEGG pathway enrichment analysis showed that targets were linked to pathways relevant to AD. The network topological analysis also revealed several high-degree nodes, suggesting key genes that may play critical roles in Alzheimer's disease pathology. Modules identified within the network correspond to known AD pathways, such as neuro-inflammation and amyloid processing, and the network is highly connected. This high level of connectivity often enhances the network's functionality and resilience.

This current research supports the existing interconnection between the mTOR signaling pathway, the Autophagy mechanism, and AD [49]. An intricate network of molecular signal transduction pathways interacts in the pathogenic process of AD. Autophagy is a catabolic process by which cells degrade and recycle their components. It involves the formation of double-membrane vesicles called autophagosomes that engulf cytoplasmic material, including damaged organelles and proteins. These autophagosomes fuse with lysosomes to form autolysosomes, where the contents are degraded and recycled [50]. The pathophysiology of AD involves upregulation of the mTOR signaling pathway, which plays an essential role in Autophagy regulation and also other associated mechanisms (depicted in Fig. 12). The mTOR pathway negatively regulates autophagy [51]. Impaired autophagy is linked to

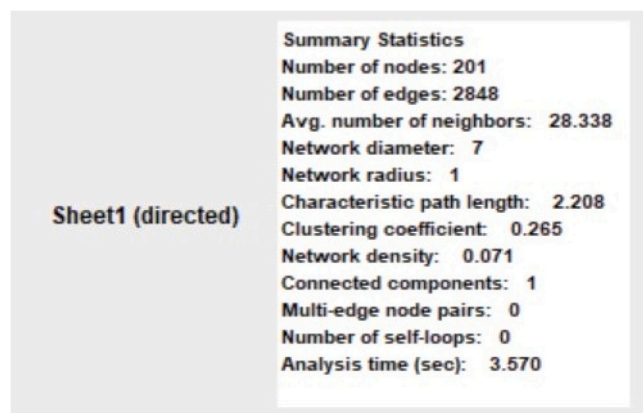


Fig. 9. Metrics of PPI network analysis performed using Cytoscape.

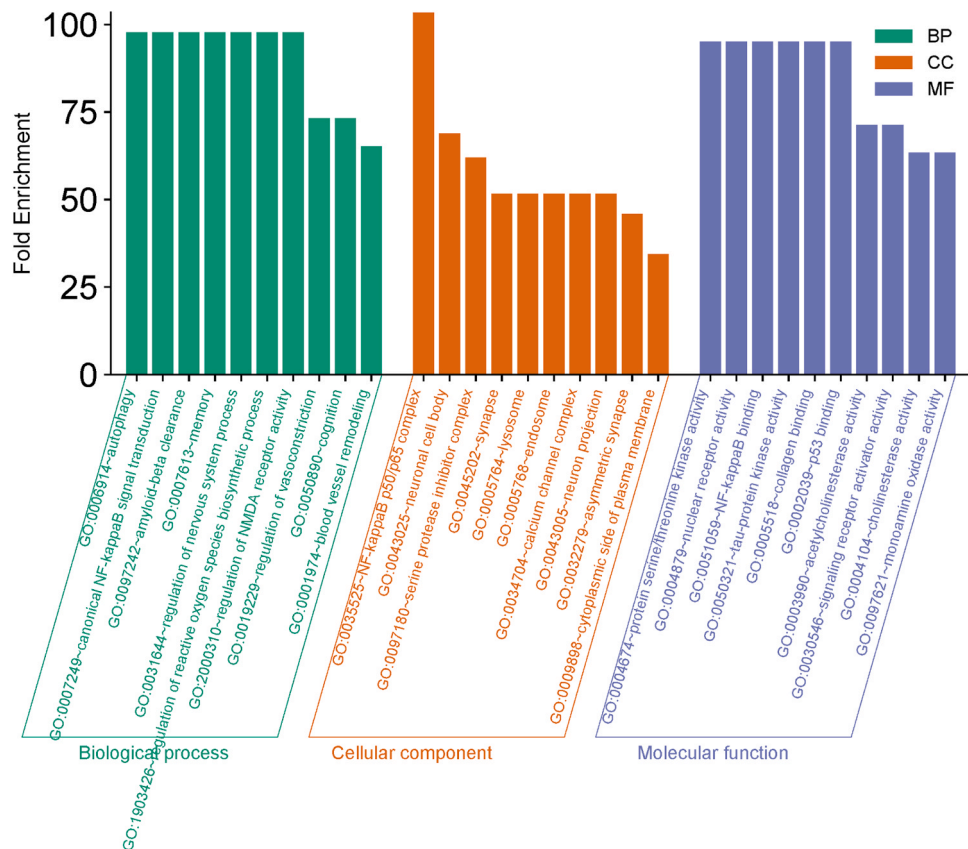


Fig. 10. Target protein GO enrichment analysis. The quantity of GO entries ($P < 0.05$) in the functional categories of biological process (BP), molecular function (MF), and cell composition (CC).

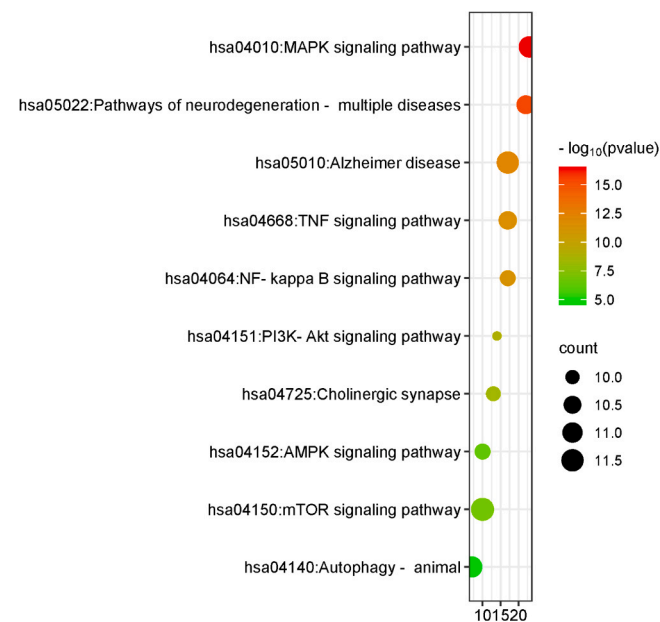


Fig. 11. Top 10 KEGG terms of hub genes.

the accumulation of protein aggregates in diseases like AD. Modulating mTOR and autophagy may offer therapeutic benefits against the disease. The mTOR signaling system and autophagy are inextricably linked.

A growing body of scientific evidence suggests that polyphenols usually have a neuroprotective effect on AD pathology. Some of them usually act by the anti-oxidant potential to combat the free radicals

which in turn results in the inhibition of apoptosis and also helps in regulating the autophagy mechanism [52,53]. The current study meticulously explains the association between certain polyphenols and the mTOR signaling pathway and the etiology of AD, offering an analytical framework for the use of polyphenols for the management of AD [54,55]. Still, several other pathways need to be investigated in future studies.

5. Conclusion

Alzheimer's disease has substantial financial implications and is the second most common cause of death and disability globally. Despite the vast amount of research exploring multiple paths leading to AD and increasing awareness of the pathogenesis of the disease, the difficulty of translating research into clinical settings has significantly hampered developments in AD research. Our study has shed light on the possible interaction between certain polyphenols and the mTOR pathway, through which they can influence the regulation of the Autophagy mechanism, providing betterment against AD. Compared to single natural or pharmaceutical chemical substances, significantly more effective outcomes can be acquired in a shorter amount of time by ingesting foods or natural items with an increased and diverse level of polyphenols (polyphenol cocktail). Therefore, further research is needed in the future to explore other pathways and genes to provide in-depth knowledge in the prevention of AD and also laboratory experiments are warranted to further explore the pharmacological potential of the shortlisted polyphenolic compounds and their derivatives with a focus on developing a promising drug candidate against AD.

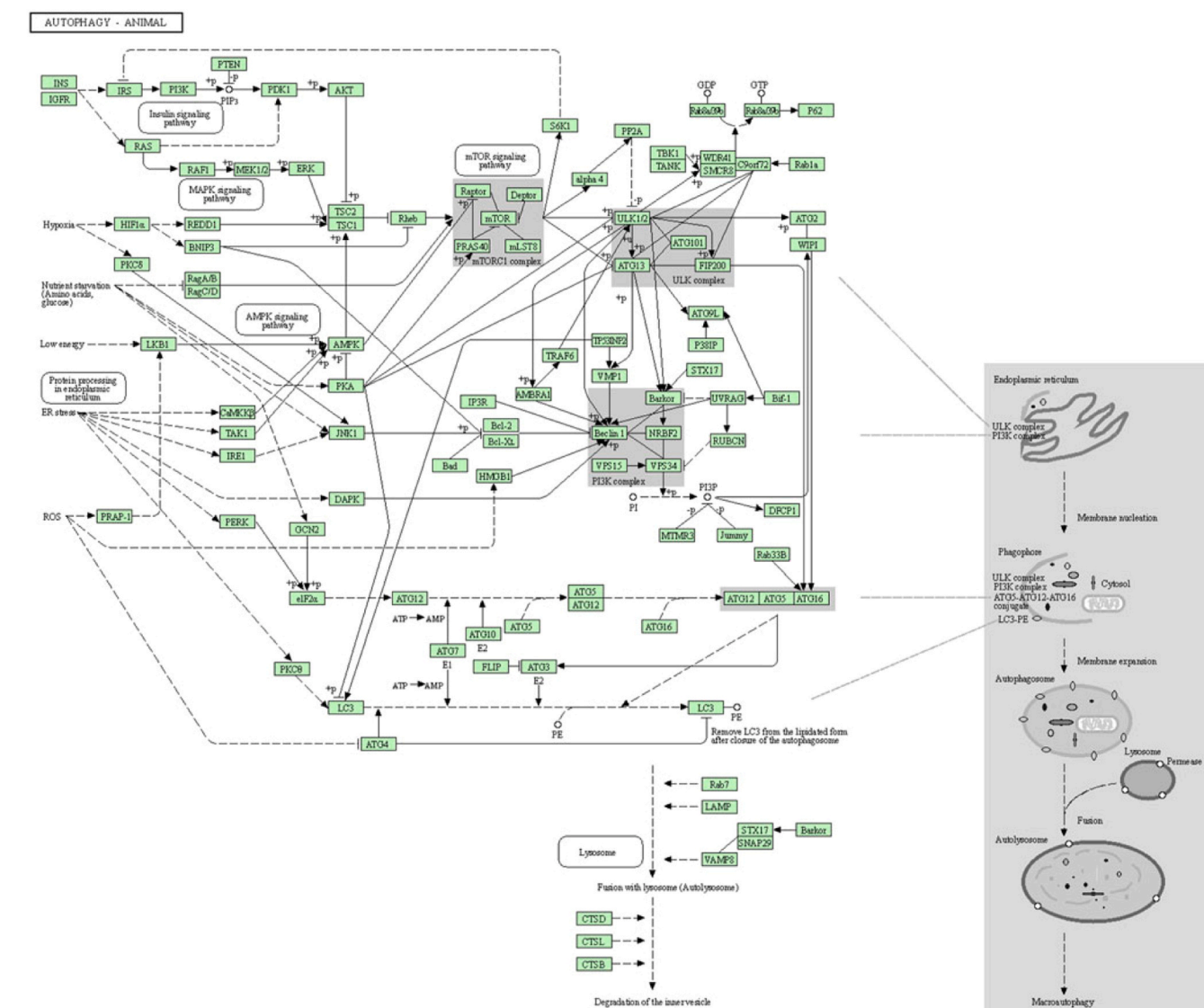


Fig. 12. Autophagy pathway (KEGG Id: hsa04140), highlighting its association with the mTOR signaling pathway (KEGG map)[43].

CRedit authorship contribution statement

Arunkumar Subramanian: Writing – original draft, Project administration, Investigation, Data curation, Conceptualization. **T. Tamilanban:** Writing – review & editing, Validation, Project administration, Investigation, Data curation, Conceptualization. **Vetrivelan Subramaniyan:** Writing – original draft, Validation, Investigation, Data curation, Conceptualization. **Mahendran Sekar:** Writing – review & editing, Project administration, Investigation, Conceptualization. **Vipin Kumar:** Writing – original draft, Visualization, Project administration, Investigation, Conceptualization. **Ashok Kumar Janakiraman:** Writing – review & editing, Project administration, Investigation. **Saminathan Kayarohanam:** Writing – review & editing, Project administration, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.toxrep.2024.101715](https://doi.org/10.1016/j.toxrep.2024.101715).

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