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Molecular Mechanistic Insights of *Pancharavinda Choornam* in Cognitive Enhancement and Comorbidity Management in Neurodegenerative Disease

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Short Report

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Abstract

Background: *Pancharavinda Choornam* is an *Ashtavaidya* formulation traditionally recognized for its *Medhya*(cognitive enhancing) properties and is used in the management of neuropsychiatric comorbidities such as insomnia, anxiety, and depression associated with neurodegenerative disorders.

Aim of the study: This study aimed to elucidate the mechanism of action of *Pancharavinda Choornam* in cognitive enhancement and comorbidity management in neurodegenerative conditions using a network pharmacology approach.

Materials and methods: In vitro antioxidant assays were performed to assess antioxidant potential, followed by network pharmacology analysis to identify compound–target interactions and multitarget pathway modulation.

Results: The formulation demonstrated significant antioxidant activity and modulated key genes, including APP (amyloid precursor protein), TAU (microtubule associated protein tau), MAOA (monoamine oxidase A), BACE (beta site amyloid precursor protein–cleaving enzyme 1), PSEN (presenilin), and PSEN1 (presenilin 1) involved in neurodegenerative pathways.

Conclusion: *Pancharavinda Choornam* exhibits multitarget therapeutic potential and may serve as a promising candidate for the management of Alzheimers and Parkinsons diseases.

1. Introduction

Neurodegenerative diseases (NDs) constitute a heterogeneous group of progressive and irreversible disorders characterized by the gradual loss of neurons and synaptic function, leading to a decline in cognitive, motor, and behavioral abilities[1]. Alzheimer's disease (AD) and Parkinson's disease (PD) being the two most prevalent neurodegenerative diseases having shared mechanisms of neurodegeneration including oxidative stress and mitochondrial alterations[2]. The increasing prevalence of NDs has been associated with substantial morbidity and mortality, ranking them among the leading causes of death globally[3]. Despite extensive research, current therapies remain largely symptomatic, limited by blood–brain barrier restrictions and drug-related adverse effects[4].

This underscores the need for safer, multi-targeted therapeutic approaches capable of addressing the complex pathophysiology of neurodegeneration as well as drug-delivery challenges in neurodegenerative diseases. *Pancharavinda Choornam* (PC) is one such Ayurvedic formulation traditionally used in the management of cognitive dysfunction and neuropsychiatric comorbidities such as insomnia, depression, and anxiety. *Pancharavinda Choornam* is an empirically derived Ayurvedic formulation, adapted from *Pancharavinda Ghrita* described in the *Ashtangahrudayam*[5] and developed based on experiential clinical wisdom (*Ashtavaidya* tradition). *Ashtavaidyas* are regarded as scholarly physicians of South India (Kerala), traditionally well versed in the eight classical branches of Ayurveda[6]. The formulation contains five parts (stem, tuber, petals, seed and stamen) of the lotus plant *(Pancharavinda)* and is traditionally

administered with ghee or milk as adjuvants. Despite its extensive traditional usage, the molecular and mechanistic basis of PC in neurodegenerative disease management remains inadequately understood. Network pharmacology has emerged as a powerful systems-level approach capable of elucidating multi-component, multi-target interactions, thereby aligning well with the holistic principles of Ayurveda[7]. This approach enables the identification of key therapeutic targets and pathways involved in neurodegeneration, including amyloid-β aggregation, tau protein phosphorylation, α-synuclein accumulation, oxidative stress, synaptic dysfunction, neurotransmitter imbalance, and impaired autophagy.

In this context, the present study employs in vitro antioxidant evaluation and network pharmacology analysis to explore the mechanistic basis of PC in cognitive enhancement and neurodegenerative disease management, providing scientific validation for its traditional therapeutic claims.

2. Materials and Methods

DPPH antioxidant assay

The antioxidant activity of *Pancharavinda Choornam* (Vaidyaratnam Oushadhasala; Batch No. 24A1278) was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Two grams of the powdered formulation were extracted with 25 ml ethanol by continuous shaking at room temperature for 24 h and filtered through Whatman No. 1 filter paper.

A DPPH stock solution was prepared by dissolving 24 mg of DPPH in 100 ml of ethanol. For the assay, 5 ml of DPPH solution was mixed with graded concentrations of the extract (10–30 mg/mL; **Table 1**), while ethanol served as the control. After incubation in the dark for 30 min, absorbance was measured at 517 nm. Antioxidant activity (%) was calculated using the formula given below[8] and expressed as equivalent antioxidant capacity[9] (**Supplementary Fig.1).**

% of antioxidant activity= [(Ac−As) ÷Ac] × 100

where: Ac= absorbance of the control reaction; As= absorbance of the test sample.

Phytochemical screening and ADME profiling

Phytochemical compounds were identified through database searches of Dr. Duke's Phytochemical and Ethnobotanical Database[10] (https://phytochem.nal.usda.gov) and the IMPPAT database—Indian Medicinal Plants, Phytochemistry, and Therapeutics[11] (https://cb.imsc.res.in/imppat/home). This was complemented by an extensive literature survey focusing on reported LC–MS and GC–MS profiles of different parts of *Nelumbo nucifera* Gaertn.[12–15] The retrieved phytochemicals were subsequently subjected to ADME profiling and drug-likeness evaluation.

Compound and disease target prediction

Compound targets were retrieved using SwissTargetPrediction[16](http://www.swisstargetprediction.ch/), the Similarity Ensemble Approach[17] (https://sea.bkslab.org/), and SuperPred[18] (https://prediction.charite.de/**).**

Disease-associated targets were retrieved from the GeneCards database[19] (https://www.genecards.org) and the Online Mendelian Inheritance in Man database[20] (https://omim.org).

Protein-Protein interaction and Compound target network analysis

The overlapping disease and compound targets were imported tothe STRING platform (v.12.0) to construct a protein–protein interaction (PPI) network, with the species limited to *Homo sapiens* and with highest confidence score ≥0.9 which were visulaized using Cytoscpe (v.3.10.3). MCODE Cytoscape plugin was utilized for identifying highly interconnected functional clusters relevant to neurodegenerative disease pathway.

Neurodegenerative disease–related pathways were analyzed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and Gene Ontology (GO) enrichment analysis through the Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.8. Enrichment results with $P < 0.05$ were considered statistically significant. SRplot, an online data visualization platform, was used for graphical representation of the enrichment results.

3. Results and Discussion

***Pancharavinda Choornam* (PC)**, a traditional formulation derived from five parts of *Nelumbo nucifera Gaertn.*, has been traditionally used in the management of cognitive impairment and associated neuropsychiatric conditions[5,6]**.** Based on this traditional relevance, the present study investigated the **antioxidant potential** of PC and elucidated its **mechanistic basis using a network pharmacology approach**.

The antioxidant activity of PC, evaluated using the DPPH radical scavenging assay, showed a scavenging efficiency of 91.16% with an IC_{50} of 21.6 mg/mL, indicating moderate antioxidant potential. Clinically, PC is administered with **lipid-based adjuvants**, such as ghee or milk, to enhance bioavailability and facilitate brain-targeted delivery. Supporting this approach, Bali et al. have highlighted the role of **lipid-based adjuvants and *Rasayana* formulations** in improving phytoconstituent bioavailability and central nervous system penetration[21].

Network pharmacology analysis provided mechanistic insights into the neuroprotective potential of PC. Phytochemical profiling identified 170 compounds across five parts of N. nucifera, subsequently filtered to 38 bioactive candidates based on ADME and drug-likeness criteria **(Table 2)**. Target prediction yielded **487 compound-related targets** and **1,044 disease-associated targets**, of which **244 overlapping genes** were identified as potential therapeutic targets.

Protein–protein interaction analysis and cytoHubba ranking identified **10 key hub genes such as AKT1, TNF, EGFR, STAT3, CASP3, SRC, JUN, HSP90AA1, ESR1, and HIF1A,** that play critical roles in neurodegenerative pathology.

These genes collectively regulate major pathogenic processes, including neuroinflammation (TNF, STAT3, SRC, HIF1A)[22–25], apoptosis and neuronal loss (CASP3, JUN, AKT1)[26–29]and protein misfolding with synaptic dysfunction (HSP90AA1, EGFR)[30,31].

Furthermore, MCODE analysis identified 10 functional clusters, with the most significant cluster (MCODE score = 14.125) comprising 17 nodes and 113 edges, indicating strong functional interconnectivity. Clusters with MCODE scores > 3 were considered functionally significant, and four such clusters are illustrated in **Fig. 1.**

KEGG pathway enrichment analysis (**Sankey plot, Fig. 2**) demonstrated significant enrichment of neurodegeneration-related pathways ($p < 0.05$), particularly those associated with neurodegeneration–multiple diseases, Alzheimer's disease, and Parkinson's disease. The KEGG pathway visualization (**Supplementary Fig. 2&3**) highlighted modulation of key disease-associated genes, including APP, TAU, MAOA, BACE1, PSEN, and PSEN1, suggesting that PC may influence critical molecular events involved in neurodegenerative progression.

In conclusion, these findings provide preliminary evidence supporting the **multitarget neuroprotective potential** of *Pancharavinda Choornam* and substantiate its traditional use in cognitive and neuropsychiatric disorders. These promising findings warrant further comprehensive investigation of the underlying mechanisms of action using in vitro, in vivo, and randomized controlled trial approaches.

Abbreviations

PC, Pancharavinda Choornam; APP, Amyloid Precursor Protein; TAU Microtubule-associated Protein Tau; MAOA, Monoamine Oxidase A; BACE,

Beta site amyloid precursor protein–cleaving enzyme 1; PSEN, presenilin; and PSEN1, presenilin1; NDs, Neurodegenerative diseases; AD, Alzheimer's disease; PD, Parkinson's disease; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ADME , Absorption, Distribution, Metabolism and Excretion; AKT1, Protein kinase B (RAC alpha serine/threonine-protein kinase); TNF, Tumor Necrosis Factor; EGFR, Epidermal Growth Factor Receptor; STAT3, Signal Transducer and Activator of Transcription 3; CASP3, Caspase-3; SRC, Proto Oncogene Tyrosine-Protein Kinase Src; JUN, Transcription Factor AP-1 (c-Jun); HSP90AA1, Heat Shock Protein 90 Alpha family class A member 1; ESR1, Estrogen Receptor Alpha; HIF1A, Hypoxia Inducible Factor 1 Alpha.

Declarations

Author contributions

RVM conceptualized the study, designed the methodology, performed data analysis, and prepared the manuscript draft; AGR contributed to methodology design, data interpretation, and manuscript review; PIC, VW, DM conducted the in-vitro experiments; JYN and AA was involved in study conceptualization; SKB contributed to conceptualization and manuscript review. All authors reviewed and approved the final manuscript.

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Conflict of interest

The authors affirm that there are no conflicts of interest to disclose.

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Tables

Table 1 Antioxidant activity of PC with DPPH technique.

S. No	Concentration (mg/ml)	Absorbance (A)λ at 515 nm	Antioxidant activity (%)
Control	Control	0.965	
Sample1	10	0.713	26.12
Sample2	15	0.645	33.16
Sample3	20	0.505	47.62
Sample4	25	0.390	59.52
Sample5	30	0.323	66.49

Table 2. List of identified compounds along with their corresponding PubChem Compound Identifiers (CIDs).

List of Compounds	Compound ID
6"-o-acetyldaidzin	156155
6"-o-acetylglycitin	10228095
8-prenylnaringenin	480764
Anonaine	160597
Apigenin	5280443
Apigenin 7-O-glucuronide	5319484
Cosmosiin	5280704
Astilbin	119258
Asimilobine	160875
D-catechin	9064
Diosmetin	5281612
Dehydronuciferine	821347
Ecliptasaponin d	102004874
Epicatechin	72276
2-hydroxy-1-methoxyaporphine/Floribundine	5319512
Ganoderic acid f	23247895
Hyperoside	5281643
Isoliensinine	5274591
Isoquercetin	5280804
Kaempferol	5280863
Kaempferol 3-(6"-ethylglucuronide)	44258915
Liriodenine	10144
Luteolin-7-glucoside	5280637
Lupeol	259846
Morin	5281670
N-methylasimilobine/O-Nornuciferine	197017

N-nornuciferine	12313579
Neferine	159654
Nepitrin	120742
Nuciferine	10146
Patuletin	5281678
Peoniflorin	442534
Phellibaumin a	54581651
Daucosterol	5742590
Chrysoeriol 7-o-glucoside	11294177
Tricin	5281702
Roemerin	119204
Trifolin	5282149

Figures

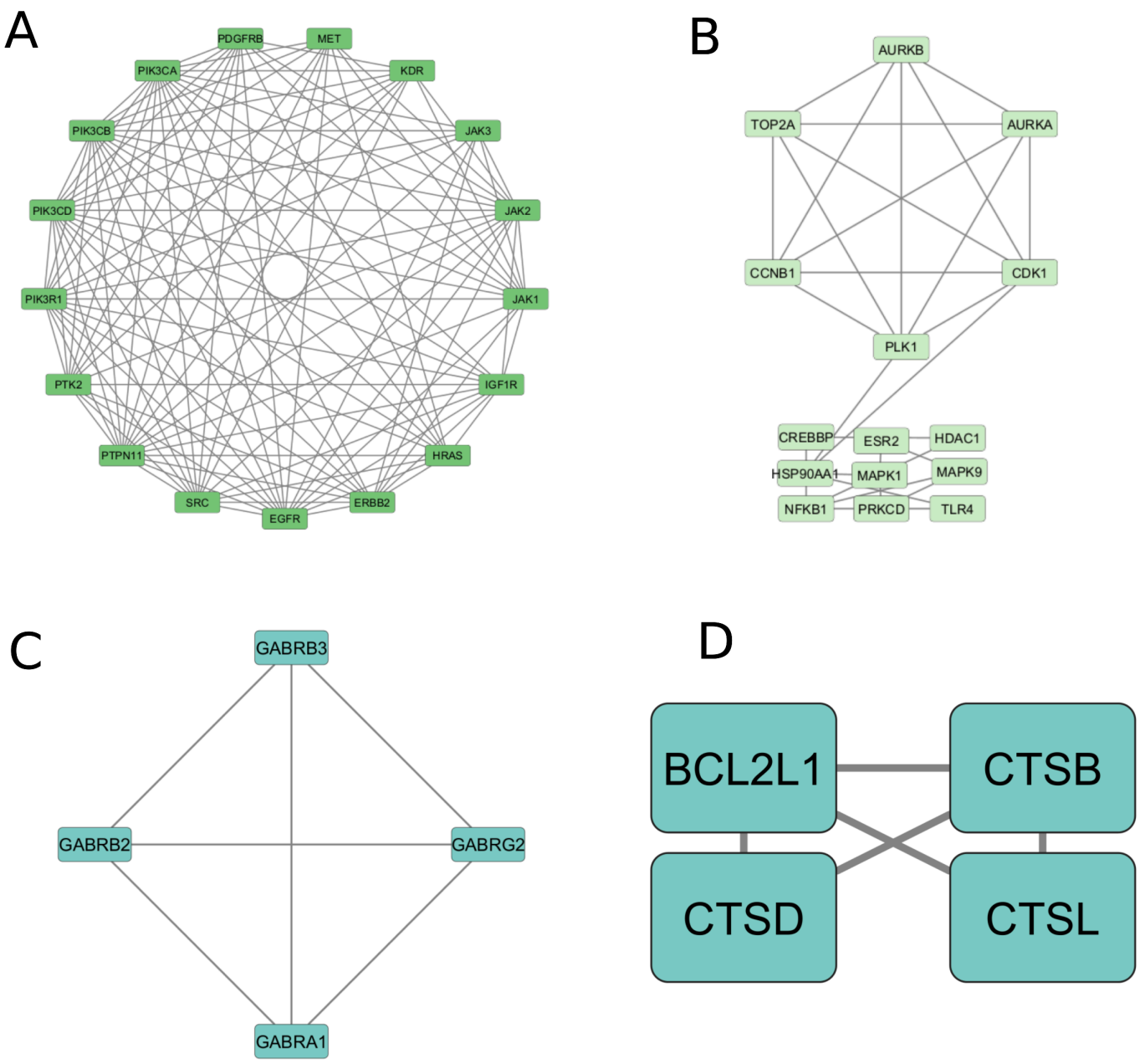


Figure 1

MCODE clusters with an MCODE score > 3, demonstrating higher functional association among the clustered genes. **A.** MCODE cluster of receptor tyrosine kinase–mediated PI3K–AKT, JAK–STAT, and MAPK signaling pathways associated with neurodegenerative diseases (MCODE Score- 14.125). **B.** MCODE cluster of hub genes involved in NF-κB signaling, cell-cycle dysregulation, epigenetic control, and MAPK-mediated neuronal stress responses in neurodegeneration (MCODE Score- 4.000). **C.** MCODE cluster representing GABA_A receptor subunits involved in inhibitory neurotransmission dysregulation associated with neurodegeneration (MCODE Score- 4.000). **D.** MCODE cluster highlighting lysosomal cathepsins and apoptosis regulators involved in impaired protein clearance and neuronal survival in neurodegeneration (MCODE Score- 3.333).

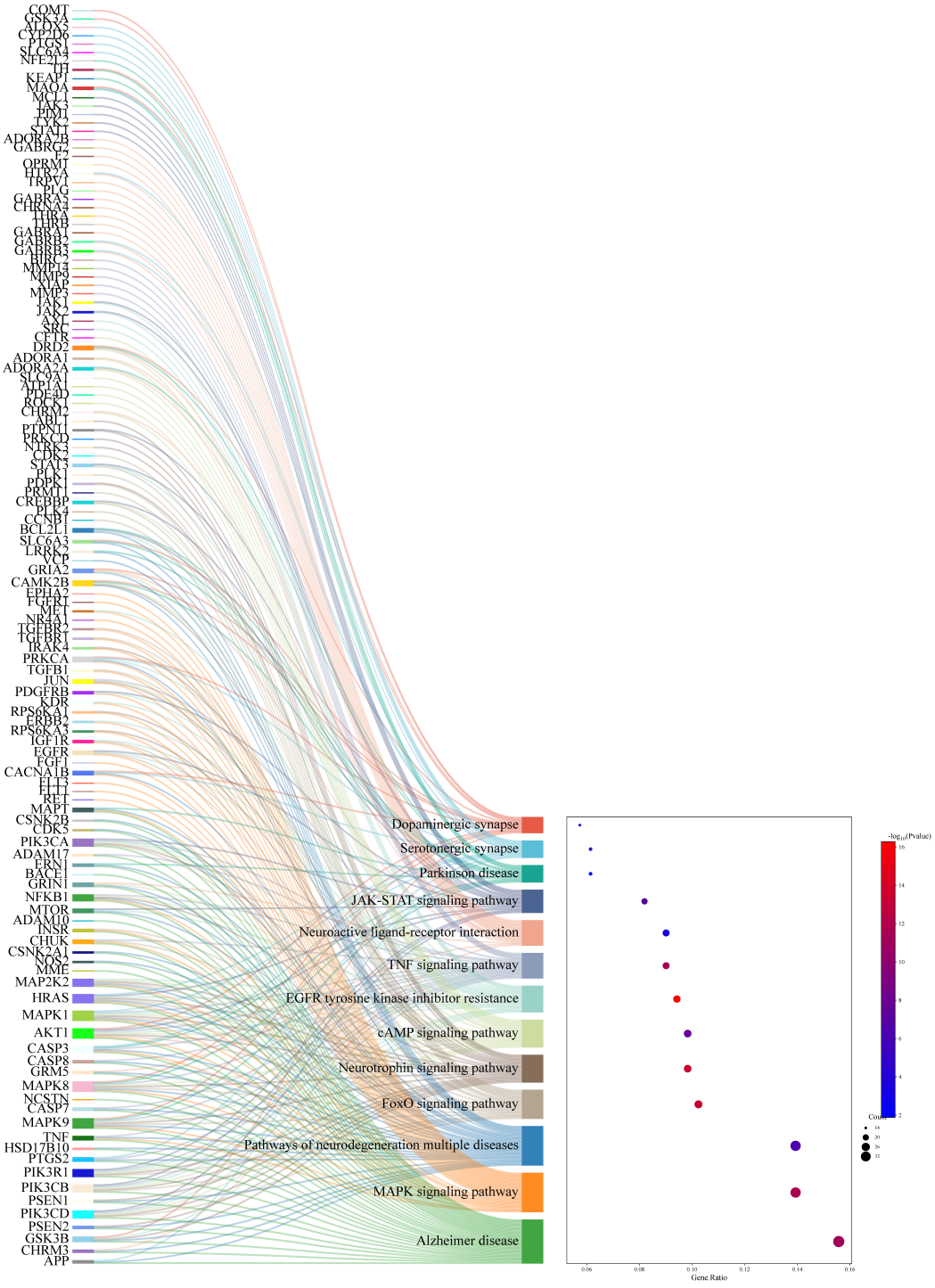


Figure 2

Sankey plot depicting target gene–pathway associations in KEGG pathway enrichment analysis.

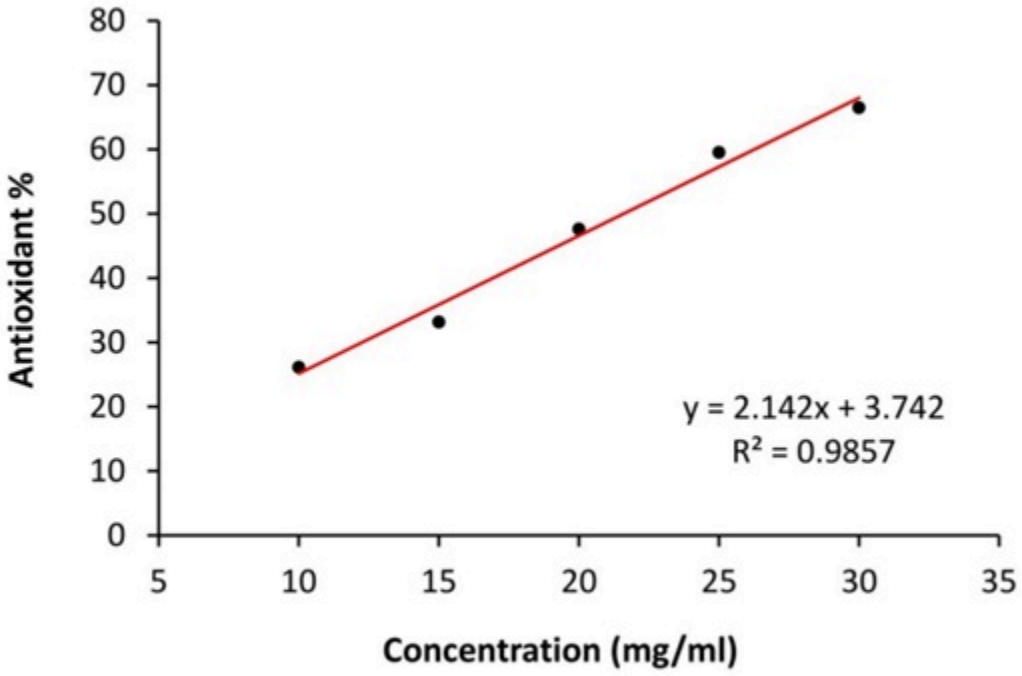


Figure 3

Linear relation between concentration and % antioxidant activity determined by absorbance at 517nm for various concentrations.

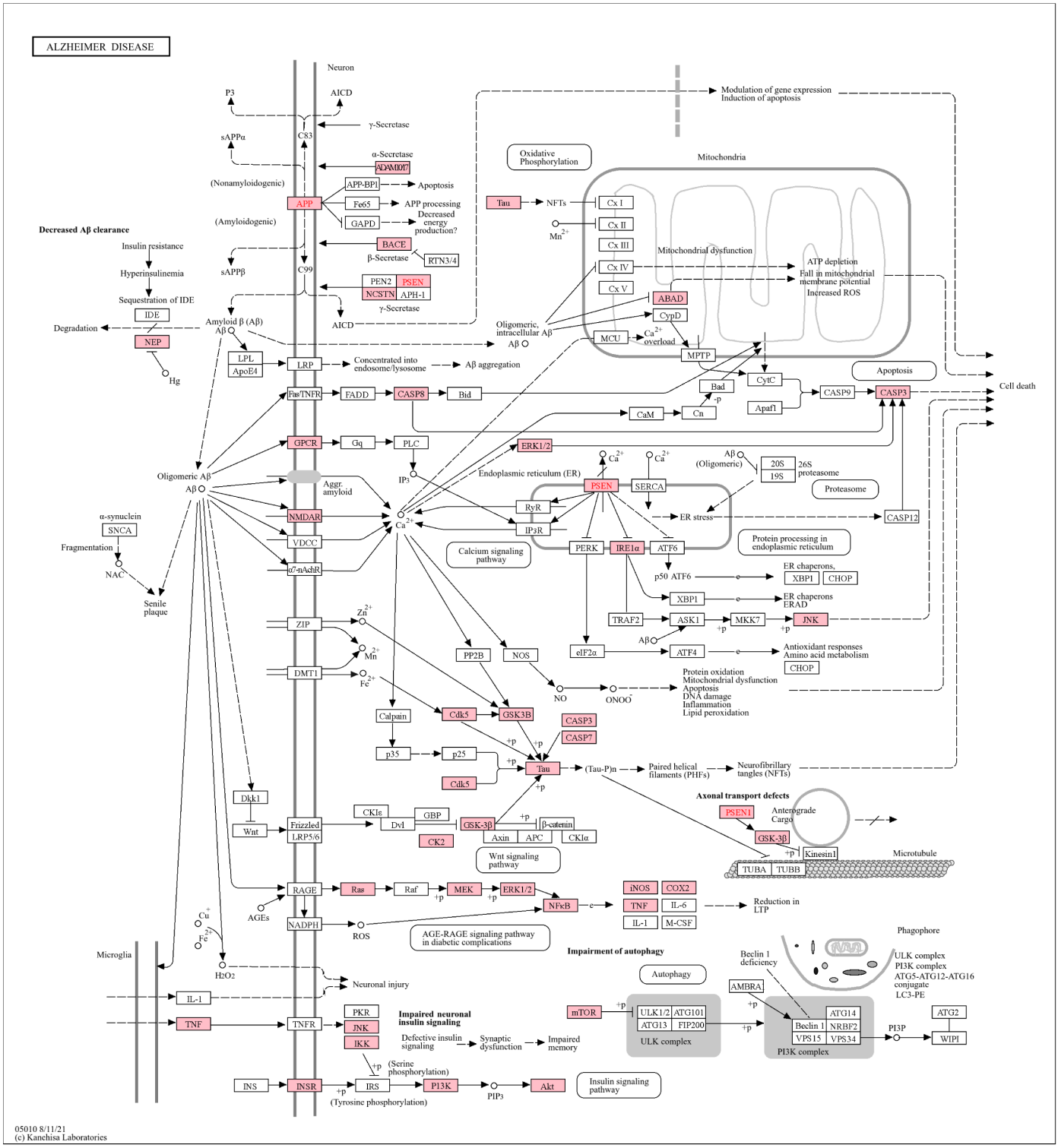


Figure 4

KEGG pathway representation of Alzheimer's disease–associated molecular mechanisms, with enriched genes indicated in pink.

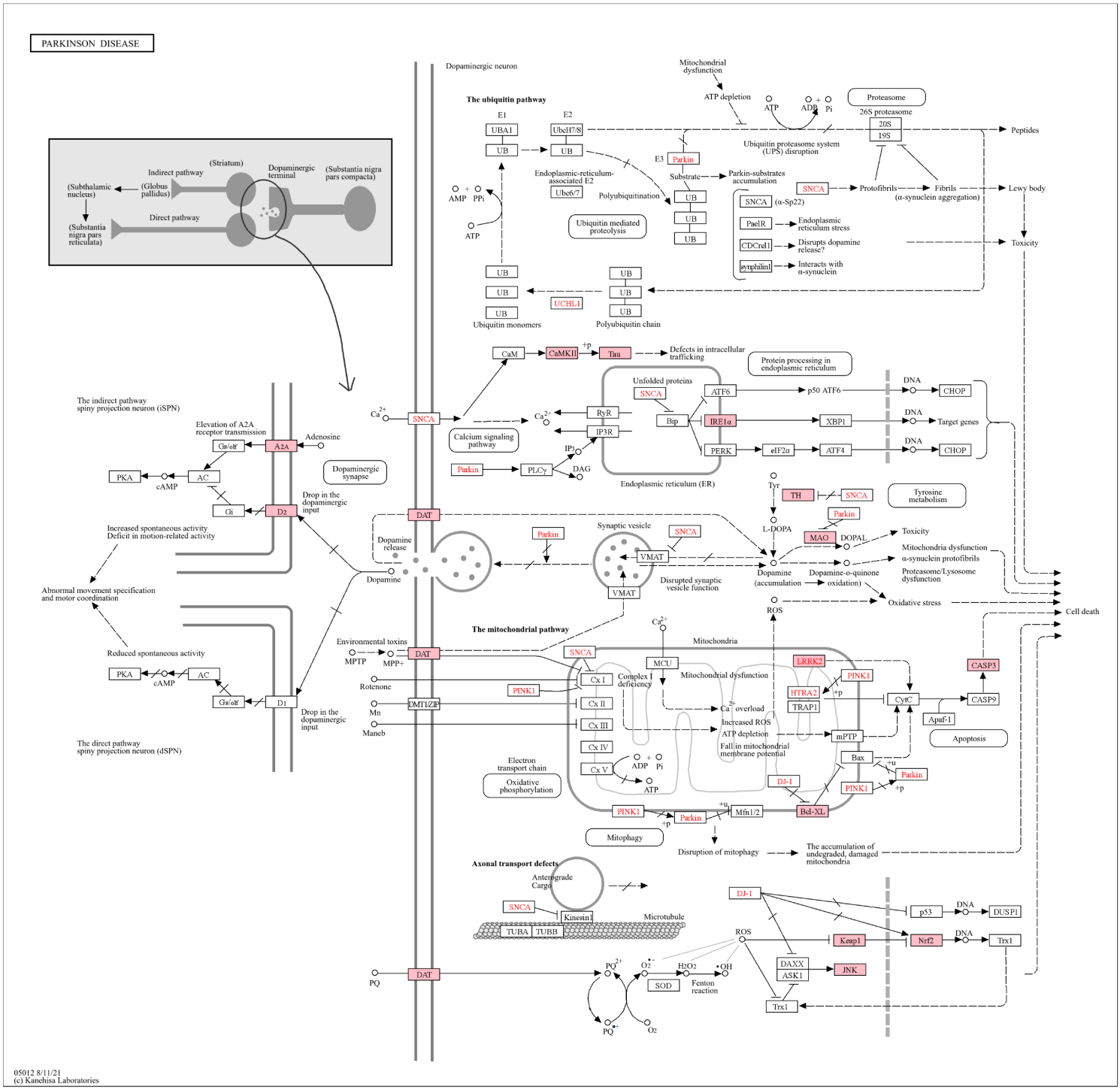


Figure 5

KEGG pathway representation of Parkinsons disease–associated molecular mechanisms, with enriched genes indicated in pink.