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In silico Analysis and Antioxidant Activity of Flavonoids from *Tinospora cordifolia* Targeting α-Synuclein of Parkinson's Disease

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This study investigates the neuroprotective potential of flavonoid-rich *Tinospora cordifolia* extract in targeting α-synuclein using a multidisciplinary approach combining *in silico*, network pharmacology and antioxidant analyses. Ethanolic extract of *T. cordifolia* was subjected to GC-MS analysis, identifying 130 phytocompounds. These compounds were screened for drug-likeness and pharmacokinetics using Lipinski's rule of five and ADMET profiling. Network pharmacology identified hub genes such as SNCA, PARK7 and LRRK2, confirming the relevance of selected targets to Parkinson's disease. Molecular docking studies were conducted against α-synuclein protein (PDB ID: 3Q27), revealing that N,1-dimethyl-4-piperidinamine, exhibited the highest binding affinity (-7.32 kcal/mol), outperforming the standard drug levodopa (-5.98 kcal/mol). Antioxidant potential of *T. cordifolia* was assessed using DPPH and FRAP assays. The DPPH assay showed strong radical scavenging activity, with up to 85% inhibition at 500 µg/mL, surpassing the standard quercetin. In contrast, FRAP results indicated lower ferric reducing power (~25%), suggesting selective antioxidant mechanisms. The results demonstrate that *T. cordifolia* contains bioactive compounds with promising neuroprotective, antioxidant and pharmacological properties that could be leveraged for multi-targeted intervention in Parkinson's disease. These findings support further investigation and development of *T. cordifolia*-based therapeutic candidates.

Keywords: Parkinson's disease, Network pharmacology, Molecular docking, Antioxidant activity.

INTRODUCTION

Parkinson's disease is a condition in which brain cells responsible for controlling movement gradually deteriorate over time and affect motor skills functions like tremors and stiffness, in the body's posture balance and control [1]. The physical signs are often accompanied by symptoms beyond movement difficulties, including impairments in thinking and memory as well as disruptions in sleep patterns and bodily functions, which can significantly affect patients' daily experiences [2]. The etiology of Parkinson's disease is multi-factorial, brought about by complex interactions between genetic and environmental causes [3]. Though familial forms are well-recognized, most Parkinson's disease are sporadic in nature, with advancing age, male gender and exposures to certain neurotoxicants as known risk factors [4]. It is paralleled by the accumulation of α-synuclein protein aggregates-so-called Lewy bodies within neurons following the course of the pathogenesis of Parkinson's disease, which in turn results in neuronal death and thus a reduction of dopaminergic signaling within the brain [5-8].

The SNCA gene, which encodes the α-synuclein protein, is a key factor in neurodegenerative disorders, particularly Parkinson's disease and other synucleinopathies [9]. α-Synuclein, a presynaptic neuronal protein, plays a vital role in the pathophysiology of these diseases. Its involvement is highlighted by genetic mutations and structural variants affecting its expression and function [10]. Initially identified as a precursor to the non-β-amyloid component in Alzheimer's plaques, its importance grew with the discovery of mutations in familial Parkinson's cases. SNCA's role is further supported by point mutations and genomic multiplications linked to autosomal-dominant parkinsonism [11,12]. Flavonoids, known for their neuroprotective properties, help combat oxidative stress, inflammation and neuronal death, offering therapeutic potential in diseases like Alzheimer's, Parkinson's and Huntington's [13].

Tinospora cordifiolia or Guduchi or Amrita is one of the most valued medicinal plants used in Ayurveda, Siddha and

Unani system of Indian traditional medicines. It is claimed to have numerous medicinal uses and belongs to the Menispermaceae family. The plant is traditionally prescribed for the treatment of conditions such as diabetes, leprosy, fever, skin disorders, diarrhoea and urinary diseases [14]. Alkaloids, terpenoids, lignans, steroids and flavonoids form a diverse arsenal of phytochemical agents in *T. cordifolia*, contributing to its wide range of pharmacological effects, which include antitumor, anti-inflammatory, immunomodulatory, anticancer, antidiabetic, antibacterial and antioxidant properties, among others [15]. Extracts from the plant have also been reported to inhibit lipid peroxidation and clear superoxide anions and hydroxyl radical, thereby preventing oxidative damage within the biological systems [16,17]. Following this potent antioxidant activity of *T. cordifolia* is of biological interest due to the multitude of beneficial phytochemicals contained in the plant. In particular, this significant contribution is perhaps due to the phenolic constituents that trigger the stimulation of superoxide dismutase, catalase and glutathione peroxidase [18,19]. *T. cordifolia* also differentially elicited gene expression involved in antioxidant defense mechanisms to substantiate its role in oxidative stress reduction and forward it as an important candidate in studying the therapeutic possibilities for the conditions associated with diabetes and neurodegeneration [20].

Flavonoids present in *T. cordifolia* play a vital role in its medicinal efficacy, particularly through their potent antioxidant activity. These compounds help neutralize free radicals and reduce oxidative stress, an underlying factor in various neurodegenerative conditions. In the context of Parkinson's disease, where oxidative stress significantly contributes to neuronal damage, the antioxidant properties of *T. cordifolia* flavonoids are especially important in supporting its neuroprotective potential [21].

EXPERIMENTAL

Extraction of *T. cordifolia*: *T. cordifolia* was extracted using three different solvents in successive extraction processes involving ethanol, hexane and water. Ethanol extraction involved digestion of 20 g of coarsely powdered sample with 70% ethanol in a volume ratio of 1:5 overnight at room temperature. The extract was filtered on Whatman No.1 filter paper and the residue stored. Hexane extraction was performed by treating 10 g of the powdered sample with hexane at room temperature overnight. The mixture was then filtered using Whatman No. 1 filter paper and the residue was collected and stored. An aqueous extract was obtained by shaking a cold mixture of 10 g of powdered sample with 100 mL distilled water overnight at room temperature and filtered on Whatman No.1 filter paper, supernatant stored [22].

GC-MS analysis: The GC-MS was done using the Perkin-Elmer Clarus 680 and 600 gas chromatograph to analyse the ethanol extracts of *T. cordifolia*. The temperature of the oven was initially set at 50 ºC and the injection temperature was 250 ºC at an intensity of 10 ºC/min and the carrier gas used helium with an average flow rate of 1 mL/min. The sample was injected using the split sampling method with the split ratio index of 40.0 and carrier gas saver split ratio of 5.0. The duration of retention indicators (RI) of the phytochemicals were calculated by comparing them to the retention periods of a series and the identity of each component was validated by comparing its retention index to those of legitimate compounds or data from the literature [23]. Mass-spectrum was interpreted using the National Institute of Standards and Technology (NIST) database. The spectra of unidentified elements have been contrasted to that of known components contained in the NIST database. The names, molecular formulas, molecular weights and chemical structures of the test compounds were determined. The peak in the GC-MS of the extract of *T. cordifolia* indicated the existence of secondary phytochemical compounds such as alkaloids, flavonoids, alcohols as well as few esters [24].

Protein protein interaction network: A protein-protein interaction (PPI) network was develoed using STRING (search tool for the retrieval of interacting genes/proteins) and visualized with Cytoscape software to elucidate the molecular underpinnings of Parkinson's disease (PD) and pinpoint critical treatment targets. STRING database (https://string-db.org) with high-confidence interaction parameters (score ≥ 0.7) pertinent to Homo sapiens. The resulting interaction network emphasised both direct and indirect interactions among proteins implicated in essential pathways, including mitochondrial dysfunction, oxidative stress, protein degradation and synaptic signalling. The network was exported and subsequently loaded into Cytoscape (v3.7.1) for visualization and topological analysis [25]. Network analyzer was employed to evaluate node degree and centrality, identifying hub proteins that are crucial in illness progression. Subsequent enrichment analysis with GO and KEGG annotations elucidated the biological processes and molecular activities governed by this protein [26].

GO and KEGG pathway annotation: Scientists analyze molecular pathways using databases like the Kyoto Encyclopedia of Genes and Genomes (KEGG version 113.0). KEGG offers indepth details on metabolic and signaling pathways allowing researchers to track the biological processes that shared protein targets affect. Pathway annotation maps these targets to specific pathways to grasp their functions in cellular processes [27]. This analysis identifies key nodes and interactions within the pathways, offering insight into the potential impact of the compounds on disease progression.

Drug likeness screening: To evaluate the drug-likeness of the 130 compounds identified through GC-MS analysis, Lipinski's rule of five screening was performed. The analysis was conducted using the Supercomputing Facility for Bioinformatics & Computational Biology at IIT Delhi, India. This platform provides several key metrics, including molecular weight, hydrogen bond donors and acceptors and log P values. The PDB file of each compound was submitted with the pH set to 7. Based on Lipinski's criteria, the results indicated which molecules were most likely to possess drug-like properties [28].

ADMET screening: The SwissADME tool (http://www.swissadme.ch/) provides an analysis of the physico-chemical properties of molecular compounds, including lipophilicity, water solubility, pharmacokinetics, druglikeness and medicinal chemistry. The bioavailability characteristics of both the chosen substance and the standard were determined through

an examination of the bioavailability RADAR. Each of the selected compounds adhered to Lipinski's guideline of 500 g/mol (SIZE), indicating a positive potential for therapeutic application. The total polarity surface area (TPSA) was employed to assess polarity (POLAR), with an ideal range set between 20 and 130 $Å^2$. All selected compounds, along with the standard, fall within the acceptable TPSA limits. In evaluating the flexibility (FLEX) property, it is essential that the value for the number of rotatable bonds does not exceed nine. The parameters xlogP3 and ESOL (log S) were employed to assess lipophilicity and insolubility (INSOLU) and (LIPO), with recommended ranges of -0.7 to +0.5 and 0 to 6, respectively. The bioavailability scores of the selected substances are recorded at 0.55, which surpasses the benchmark of 0.17. Consequently, all selected compounds demonstrate outstanding characteristics for oral availability [29].

Preparation of protein: Two-dimensional (2D) structure of the target protein was extracted in PDB format from the protein data bank (PDB) (https://www.rcsb.org/), (PDB ID: 3Q27-1.27 Å). All water molecules were eliminated from the target structure using Discovery Studio Visualizer and SO4 and GOL were eliminated from 3Q27. The PDB format was then saved. The saved protein PDB file from Discovery Studio Visualizer was opened using AutoDock Tools 1.5.7 and Kollman charges were assigned, hydrogen atoms were inserted and missing residues were constructed. The protein output ready for docking was stored in PDBQT format [30].

Preparation of ligand: The canonical smiles of the ligands were obtained from PubChem, which is then converted into PDB format. Once again, the stored ligand files in PDB format were analyzed using AutoDock Tools 1.5.7 to determine the root of the ligand and assign Gasteiger charges for ligands. The PDBQT format was used to store the prepared ligand output for docking. Prior to docking, each ligand should undergo this procedure [31].

Molecular docking: The docking procedure was conducted following the methodology as outlined by Khan *et al.* [32]. For the blind docking process, the grid box was configured with specific parameters, including a spacing of 0.375 Å and dimensions set to 126 points in both the x and y directions, as well as 126 points in the z-direction. After adjusting the dimensions and confirming the grid box settings, the configuration was saved as a grid parameter file (GPF). The genetic algorithm was then established with the protein molecule treated as a rigid file, specifying 30 runs for the genetic algorithm and a population size of 300, while other parameters were remained at their default settings. The outcomes from the lamarckian genetic algorithm (LGA) were subsequently saved as a docking parameter file (DPF). The Autogrid and Autodock processes were executed utilizing the GPF and DPF files, resulting in the generation of GLG and DLG files, respectively [33].

Discovery studio: The PyMOL© Molecular Graphics System, Discovery Studio Visualizer and the Protein-Ligand Interaction Profiler's web server (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index) were utilized to illustrate the protein-ligand complexes and their molecular interactions [34].

Antioxidant activity: The antioxidant activity of *T. cordifolia* extract was assessed using two standard *in vitro* assays.

DPPH radical scavenging assay: The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to evaluate the free radical scavenging ability of *T. cordifolia* extract. This assay measures the reduction in DPPH radicals, indicated by a colour change from deep violet to pale yellow, which is quantified spectrophotometrically. The *T. cordifolia* extract was tested at varying concentrations (100, 200, 300, 400 and 500 μg/mL), with quercetin serving as the standard antioxidant for comparison. The percentage of inhibition was calculated by comparing the absorbance of the control (DPPH without extract) to that of the sample [35].

FRAP (ferric reducing antioxidant power) assay: The FRAP assay assesses the reducing power of antioxidants by measuring their ability to reduce Fe^{3+} (ferric) to Fe^{2+} (ferrous) ions. Similar concentrations of *T. cordifolia* extract (100-500 μg/mL) were used. Gallic acid served as the standard reference compound. The formation of ferrous-TPTZ complex was measured spectrophotometrically [36].

RESULTS AND DISCUSSION

GC-MS analysis: A total of 130 bioactive compounds were identified in the ethanol extract of *T. cordifolia* using GC-MS spectrum and the details are given in Table-1. The compounds, including flavonoids, alkaloids, alcohols, esters, pyrazoles and terpenoids, belong to various chemical classes, highlighting the phytochemical richness of the extract. Compounds like N,1-dimethyl-4-piperidinamine are observed for their pain-relieving, CNS-stimulating and seizure-inhibiting effects. Moreover, 1,3,5-benzenetriol and 8-hydroxy-2-octanone are also recognized for their potent antimicrobial and antioxidant properties, while β-asarone demonstrated neuroprotective and anti-inflammatory effects [37].

Antioxidant compounds like 1,3,5-benzenetriol and acetic acid derivatives help the plant fight oxidative stress, which is a major cause of neurodegeneration in Parkinson's disease [38]. These results were improved by drug-likeness screening using Lipinski's rule of five and ADMET profiling, which reduced the active candidates to a small group of molecules that could be used as drugs. Some compounds showed good pharmacokinetic qualities such as high absorption in the gastrointestinal tract and permeability across the blood-brain barrier, which makes them more useful for therapies that target the central nervous system (CNS).

Protein-protein interaction: The protein-protein interaction (PPI) network, constructed using STRING database, visualized *via* cytoscape, provides a detailed molecular interaction map relevant to Parkinson's disease. Whereas high confidence interaction data (confidence score ≥ 0.7) revealed significant interconnection among multiple proteins, including those implicated in mitochondria dysfunction, oxidative stress, autophagy and synaptic signaling-pathways critically disrupted in Parkinson's disease pathology [39] (Fig. 1). Using Cytoscape v_3.7.1, a compound-target-anxiety network can be constructed to visualize the relationships between compounds, their protein targets and anxiety-related pathways. This network reveals key interactions and hub nodes, such as neurotransmitter-related targets (*e.g.*, serotonin receptors), that are central to anxiety mechanisms [40]. The visualized PPI

TABLE-1
GC-MS RESULTS WITH THE RETENTION TIME AND PEAK AREA % DATA OF *Tinospora Cordifolia* EXTRACT

S. No.	RT	m.f.	m.w.	Compound name	Area (%)
1	3.930	$C_8H_{16}O_2$	144.21	1,1-Diethoxy-2-butene	2.03
2	4.210	$C_8H_{18}O_2$	146.23	Propane, 1,1-diethoxy-2-methyl-	0.28
3	4.542	$C_7H_{16}N_2$	128.22	4-Piperidinamine, N,1-dimethyl-	0.06
4	4.665	$C_8H_{15}N$	125.21	3-Azabicyclo[3.2.2]nonane	0.03
5	4.989	$C_6H_6D_2O$	100.15	4,4-Dideuterocyclohexa-2-en-1-ol	0.53
6	5.425	$C_6H_{10}O_2$	114.14	4,4-Dimethoxybut-1-yne	0.05
7	5.521	$C_7H_{14}O$	114.19	*cis*-2,3-Epoxyheptane	0.44
8	5.605	$C_4H_9BO_2$	99.93	1,3,2-Dioxaborolane, 2-ethyl-	0.24
9	5.660	$C_9H_{20}O_2$	160.25	Butane, 1,1-diethoxy-3-methyl-	0.26
10	5.777	$C_7H_{13}N_3$	139.20	1-(5-Methyl-1*H*-pyrazol-3-yl)-2-propanamine	0.07
11	5.914	C_9H_{18}	126.24	Cyclohexane, 1,2,3-trimethyl-, (1α,2β,3α)-	0.06
12	6.050	$C_5H_{12}O_2$	104.15	1,4-Pentanediol	0.02
13	6.110	$C_8H_{18}O_2$	146.23	1,3-Octanediol	0.06
14	6.178	$C_8H_{20}O_4Si$	208.33	Silicic acid (H_4SIO_4), tetraethyl ester	0.64
15	6.340	$C_4H_5NO_2$	99.09	Pyrrolidine-2,4-dione	0.10
16	6.607	$C_4H_8N_2O_2S$	148.18	Ethanimidothioic acid, N-[[(methylamino)carbonyl]oxy]-	0.05
17	6.668	$C_8H_{18}O_2$	146.23	1,3-Octanediol	0.06
18	6.870	$C_6H_{12}O_2$	116.16	2-Propanol, 1-(2-propenyloxy)-	0.03
19	6.961	$C_9H_{20}OSi$	176.34	Dimethyl(1-cyclopentylethoxy)silane	0.21
20	7.710	$C_9H_{20}O_3$	176.25	Propane, 1,1,3-triethoxy-	1.55
21	7.860	$C_6H_{11}N$	97.16	N,N-Dimethyl-1,3-butadien-1-amine	0.37
22	7.940	$C_6H_{11}N$	97.16	7-Azabicyclo[4.1.0]heptane	0.34
23	8.016	$C_{10}H_{20}O$	156.27	Cyclohexanepropanol, α-methyl-	0.18
24	8.099	$C_{10}H_{22}O_3$	190.28	1,1,3-Triethoxybutane	2.64
25	8.450	$C_2H_5O_5P$	140.03	Phosphonoacetic acid	0.04
26	8.724	$C_5H_8N_4S$	144.21	1*H*-Pyrazole-1-carbothioamide, 3,5-dimethyl-	0.03
27	8.800	$C_4H_8O_3$	104.10	Hydroperoxide, 1,4-dioxan-2-yl	0.07
28	8.915	$C_8H_{12}N_2O_4$	200.19	2-(3,5-Dimethyl-1-pyrazolyl)succinic acid	0.05
29	9.356	$C_7H_8O_2$	124.14	3-Cyclopentene-1-acetaldehyde, 2-oxo-	0.05
30	9.646	$C_{10}H_8$	128.17	Azulene	42.30
31	10.395	$C_4H_9NO_3$	119.12	3-Nitro-2-butanol	3.05
32	10.635	$C_8H_{10}N_4O$	178.19	2-[2-(Methoxymethyl)-2*H*-tetraazol-5-yl]pyridine	0.96
33	10.748	$C_7H_{16}N_2$	128.22	4-Piperidinamine, N,1-dimethyl-	2.14
34	11.045	$C_6H_8N_2O_4S_2$	224.27	Acetic acid, [[(5-oxo-2-thioxo-4-imidazolidinyl)methyl]th	0.07
35	11.105	$C_6H_6O_3$	126.11	1,3,5-Benzenetriol	0.05
36	11.330	$C_7H_{12}O_2$	128.17	2-Pentenoic acid, 3-methyl-, methyl ester	0.05
37	11.446	$C_8H_{10}O_4$	170.16	1*H*-Cyclopenta[*c*]furan-3(3a*H*)-one, 6,6a-dihydro-1-(1,3-dioxolan-2-yl)-, (3aR	0.35
38	12.025	$C_3H_6Cl_2$	112.99	Propane, 1,1-dichloro-	0.03
39	12.368	$C_2H_4O_2S$	92.12	Acetic acid, mercapto-	0.05
40	12.540	$C_6H_8N_8O_4$	244.18	Ethane, 1,2-*bis*[(4-amino-3-furazanyl)oxy]-	0.05
41	12.711	$C_5H_8N_2$	96.13	1*H*-Pyrazole, 3,5-dimethyl-	0.04
42	12.880	$C_4H_7N_3$	97.12	1*H*-Pyrazol-4-ylmethanamine	0.03
43	12.939	$C_{14}H_{26}O_4$	258.35	Oxalic acid, cyclohexylmethyl isohexyl ester	0.09
44	13.040	$C_7H_{13}N_3$	139.20	1-(5-Methyl-1*H*-pyrazol-3-yl)-2-propanamine	0.02
45	13.166	$C_5H_{13}NO$	103.17	1-Propanol, 3-(dimethylamino)-	0.02
46	13.308	$C_9H_{18}D_2O_2$	162.27	3-(Dideuteromethoxymethoxy)-1-octene	0.05
47	13.431	$C_5H_{10}O$	86.13	Cyclobutylmethanol	0.12
48	13.655	$C_7H_{12}O_2$	128.17	2-Pentenoic acid, 3-methyl-, methyl ester	0.04
49	13.798	$C_8H_{18}O_4Si_2$	234.39	Ethanedioic acid, *bis*(trimethylsilyl) ester	0.27
50	14.240	$C_{10}H_{16}$	136.23	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-	0.06
51	14.290	$C_5H_4N_2$	92.10	1*H*-Pyrrole-2-carbonitrile	0.03
52	14.351	$C_4H_{10}ClNO_2$	139.58	4-Aminobutanoic acid hydrochloride	0.02
53	14.597	$C_5H_{10}O_2$	102.13	2*H*-Pyran-4-ol, tetrahydro-	0.10
54	15.065	$C_{11}H_{20}N_4O_2$	240.30	3,7-Diazabicyclo[3.3.1]nonane-3-acetamide, 7-(hydroxymethyl)-1,5-dimethyl-	0.04
55	15.166	$C_{10}H_{19}ClO_2$	206.71	3-Chloropropionic acid, heptyl ester	0.19
56	15.445	$C_{12}H_{10}N_2O_3$	230.22	2-(5-Methyl-3-isoxazolyl)-1*H*-isoindole-1,3(2*H*)-dione #	0.21
57	15.501	$C_6H_{12}D_4O$	108.20	α-D4-Hexamethylene oxide	0.21
58	15.580	$C_5H_{12}O_5$	152.15	Pentitol	0.09
59	15.785	$C_9H_{20}OSi$	176.34	Dimethyl(1-cyclopentylethoxy)silane	0.06

60	15.850	$C_{13}H_{28}N_2$	212.38	N,N'-Di(2-methylpentenyl)-1,2-propanediamine	0.08
61	15.913	$C_5H_{14}OSi$	118.25	(Methoxymethyl)trimethylsilane	0.27
62	16.198	$C_9H_{20}OSi$	172.34	1-Methyl-1-(2-buty)oxy)-1-silacyclobutane	0.07
63	16.675	$C_8H_{19}ClSi$	178.77	Dimethylthexylsilyl chloride	0.07
64	16.732	$C_7H_{15}Cl$	134.65	Heptane, 1-chloro-	0.15
65	16.867	$C_{12}H_{26}S$	202.40	2-Undecanethiol, 2-methyl-	0.09
66	16.971	$C_4H_{10}O_4$	122.12	Butane-1,2,3,4-tetraol	0.05
67	17.182	$C_{18}H_{38}O$	270.49	1-Octadecanol	0.08
68	17.405	$C_{14}H_{20}O_2$	220.31	2-[(7-Methylenebicyclo[3.3.1]non-2-en-3-yl)oxy]ethylacet	0.05
69	17.554	$C_8H_{16}N_4$	168.24	1,4,5,8-Tetraazadecalin	0.05
70	17.623	$C_5H_8N_6O_2$	172.16	1,2,4-Triazino[5,6-e]-1,2,4-triazine-3,6-dione, hexahydro-	0.03
71	17.738	$C_8H_{18}O_4Si_2$	234.39	Ethanedioic acid, *bis*(trimethylsilyl) ester	0.19
72	17.975	$C_{20}H_{38}$	278.51	9-Eicosyne	0.26
73	18.070	$C_8H_{16}O_2$	144.21	8-Hydroxy-2-octanone	0.09
74	18.300	$C_5H_8D_2O_2$	106.14	*trans*-1,2-D2-1,2-Dihydroxy-cyclopentane	0.02
75	18.448	$C_{11}H_{15}NO$	177.24	2-Hydroxy-1-adamantanecarbonitrile	0.13
76	18.509	$C_{12}H_{24}O_3$	216.32	Propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhexyl ester	0.05
77	18.865	$C_8H_{20}OSi$	160.33	Ethoxydi(*tert*-butyl)silane	0.20
78	19.025	$C_{12}H_{22}N_2O_3$	242.32	N-(Cyclohexylcarbonyl)-4-morpholinecarboxamide #	0.26
79	19.146	$C_{11}H_{17}NO$	179.26	Benzenemethanol, α-[1-(ethylmethylamino)ethyl]-	0.50
80	19.205	$C_{12}H_{20}O_3$	212.29	Carbonic acid, but-3-yn-1-yl heptyl ester	0.28
81	19.368	$C_{24}H_{46}O_2$	366.62	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	1.32
82	19.465	$C_{10}H_{10}O_3$	178.18	Benzoic acid, 2-(1-oxopropyl)-	1.16
83	19.613	$C_{17}H_{34}O_2$	270.45	Ethyl pentadecanoate	1.27
84	19.713	$C_8H_{16}O$	128.21	2,3-Dimethylcyclohexanol 4	0.56
85	19.835	$C_{14}H_{22}O$	206.32	2,5-Cyclohexadien-1-one, 2,6-*bis*(1,1-dimethylethyl)-4-meth	1.85
86	19.985	$C_9H_{14}O$	138.21	2,6-Heptadienal, 2,4-dimethyl-	0.36
87	20.115	$C_{10}H_{13}NO_3$	195.22	Glycine, N-(phenylacetyl)-, methyl ester	0.19
88	20.256	$C_{11}H_{14}O_4$	210.23	Glutaric acid, but-3-yn-2-yl hex-4-yn-3-yl ester	0.09
89	20.305	$C_{10}H_{15}Br$	215.13	Tricyclo[3.3.1.13,7]decane, 1-bromo-	0.04
90	20.432	$C_8H_{16}O_2$	144.21	2-Ethylhexanoic acid	0.08
91	20.610	$C_{19}H_{38}O_2$	298.50	Heptadecanoic acid, ethyl ester	0.13
92	20.709	$C_{23}H_{42}O_2$	350.58	8,11,14-Docosatrienoic acid, methyl ester	0.20
93	20.855	$C_{29}H_{58}O_3Si$	486.86	Pentacosanoic acid, 2-[(trimethylsilyl)oxy]-, methyl est	0.36
94	20.970	$C_9H_{12}O$	136.19	Tricyclo[4.2.1.0 2,5]non-7-en-3-ol	0.12
95	21.154	$C_{16}H_{30}O$	238.41	Z,Z-8,10-Hexadecadien-1-ol	2.26
96	21.325	$C_{19}H_{36}O_2$	296.49	(Z)-Ethyl heptadec-9-enoate	3.99
97	21.546	$C_{19}H_{38}O_2$	298.50	Heptadecanoic acid, ethyl ester	0.96
98	21.674	$C_{10}H_{13}NO_8$	259.21	Tetraacetyl-*d*-xylonic nitrile	0.52
99	21.740	$C_8H_{16}O_8$	240.21	l-Gala-l-ido-octose	0.78
100	21.870	$C_{11}H_{20}$	152.28	Bicyclo[4.1.0]heptane, 7-butyl-	0.51
101	21.925	$C_{11}H_{12}N_2O_3S$	252.29	Acetic acid, 2-[[3-cyano-4-(methoxymethyl)-6-methyl-2-pyridinyl]thio]-	0.10
102	21.970	$C_{13}H_{10}Cl_2NO_3S$	346.20	Benzenesulfonic acid 3,5-dichloro-2,6-dimethyl-4-pyridyl ester	0.18
103	22.125	$C_{21}H_{46}OSi$	342.68	2-Octyldecanol, TMS derivative	0.58
104	22.200	$C_{14}H_{46}O_6Si_7$	490.11	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	0.52
105	22.296	C_5H_9NO	99.13	2-Piperidinone	0.31
106	22.465	$C_{14}H_{26}O$	210.36	2-Nonenal, 2-pentyl-	0.34
107	22.554	$C_{19}H_{36}O_3$	312.49	Glycidyl palmitate	0.29
108	22.665	$C_{11}H_{19}N$	165.28	Tricyclo[4.3.1.1(3,8)]undecan-1-amine	0.04
109	22.880	$C_8H_{13}ClHgO$	325.23	Chloro(9-oxabicyclo[4.2.1]non-2-yl)mercury	0.02
110	22.980	$C_{14}H_{24}O_2$	224.34	3-Cyclopentylpropionic acid, 1-(cyclopentyl)ethyl ester	0.03
111	23.160	$C_7H_{18}N_4$	146.24	1,4-Butanediamine, N-(3-aminopropyl)-	0.22
112	23.355	$C_8H_{17}N$	127.23	N-Ethyl-hexahydro-1*H*-azepine	0.18
113	23.435	$C_{16}H_{48}O_7Si_8$	592.08	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxa	0.33
114	23.512	$C_{20}H_{38}O_4$	342.50	Succinic acid, 2-ethylhexyl 4-octyl ester	0.07
115	23.565	$C_{13}H_{18}N_2O_4S_2$	330.42	S-3-[4-Phthalimidobutylamino]propyl thiosulfuric acid	0.03
116	23.805	$C_7H_9N_5O_2$	195.18	6-(4-Nitro-1*H*-pyrazol-1-yl)-2,3-dihydro-1*H*-1,4-diazepine #	0.18
117	23.926	$C_{16}H_{34}OSi$	270.53	1-Ethyl-1-tridecyloxy-1-silacyclopentane	0.13
118	24.128	$C_{16}H_{28}O_2$	252.39	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]me	1.27
119	24.228	$C_{16}H_{29}Cl_3O_3$	375.76	Carbonic acid, tridecyl 2,2,2-trichloroethyl ester	1.25
120	24.325	$C_6H_{12}O_2S$	148.22	3,3-Dimethoxytetrahydrothiophene	0.61
121	24.375	$C_6H_{12}O_2S$	148.22	3,3-Dimethoxytetrahydrothiophene	0.29

122	24.430	$C_6H_6ClNO_2S$	191.63	2-Chloro-6-(methylsulfonyl)pyridine	0.39
123	24.536	$C_{15}H_{30}O$	226.40	Pentadecanal-	0.92
124	24.623	$C_{12}H_{36}O_5Si_6$	424.93	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	1.53
125	24.772	$C_8H_6O_4$	166.13	1,2-Benzenedicarboxylic acid	1.29
126	24.881	$C_{24}H_{32}N_2O_2$	380.52	Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate	2.52
127	25.068	$C_{20}H_{40}O_2$	312.53	Octadecanoic acid, ethyl ester	0.98
128	25.259	$C_{16}H_{21}ClO_4$	312.79	1,2-Cyclohexanedicarboxylic acid, 3-chlorophenyl propyl ester	0.73
129	25.380	$C_{12}H_{16}O_3$	208.26	β-Asarone	0.42
130	25.477	$C_{21}H_{42}O$	310.56	Henicosanal	0.67

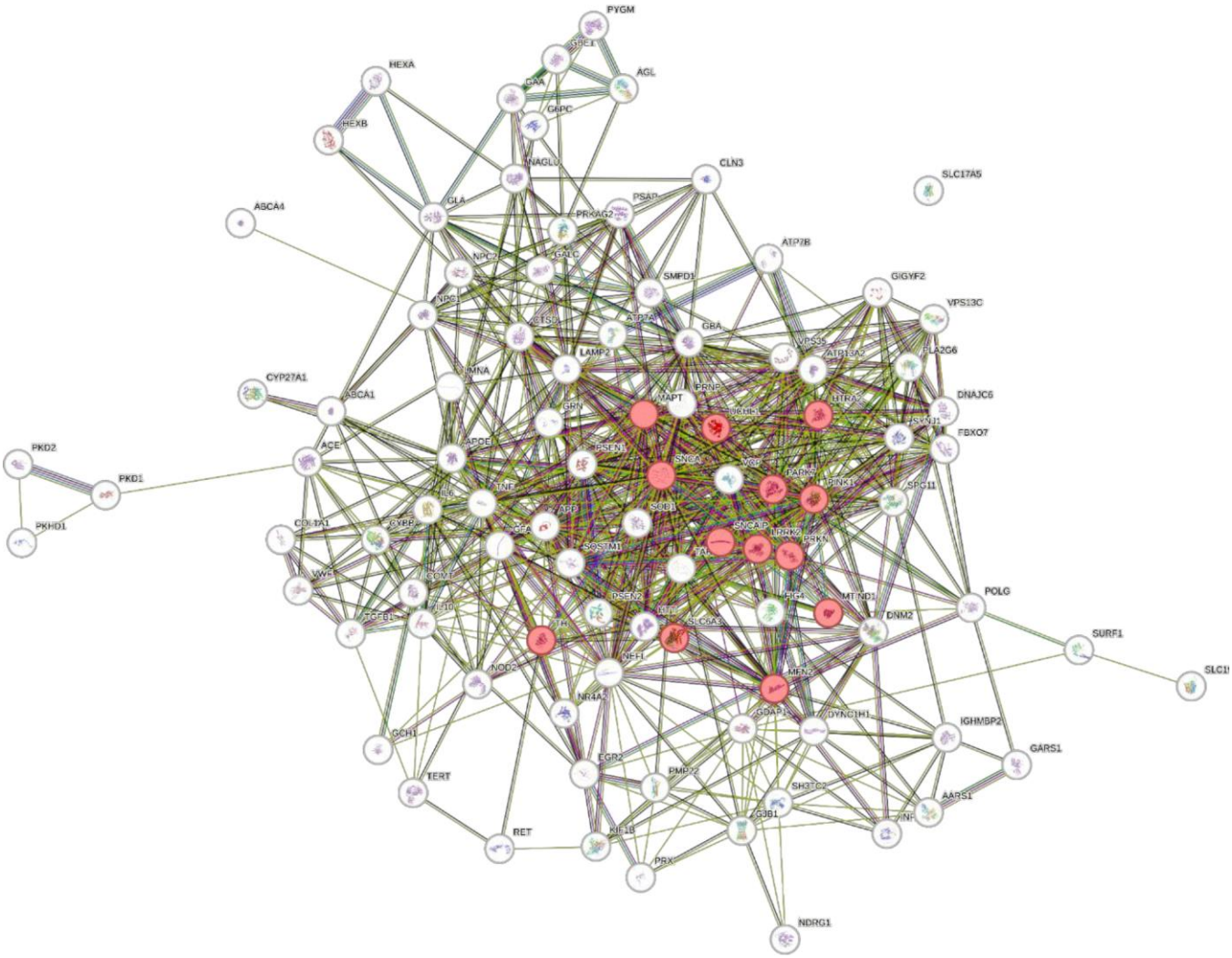

Fig. 1. String database highlighting gene's relating to Parkinson's disease

network (Fig. 2) illustrates a dense and highly interconnected system, indicating complex molecular crosstalk in Parkinson's disease. Within this network key Hub genes such as SNCA, PARK7, LRRK2 and PRKN are prominently highlighted. These genes are central nodes in the network, exhibiting the highest number of connections (degree centrality) and thus play critical roles in Parkinson's disease pathogenesis [41]. The dense interaction web suggests that these genes not only function individually but are part of complex, overlapping pathways, including oxidative stress response, autophagy, protein degradation (UPS) and neuroinflammation.

Gene enrichment analysis and KEGG pathway annotation: Gene enrichment analysis identifies the biological pathways significantly associated with a set of genes. The bar plot shows that pathways related to mitophagy, Parkinson's disease and neurodegeneration are highly enriched, with mitophagy showing the highest fold enrichment and significance (lowest FDR). Other enriched pathways include ubiquitin-mediated proteolysis, protein processing in the endoplasmic reticulum and amyotrophic lateral sclerosis. This suggests that the gene set is strongly linked to cellular stress responses, mitochondrial quality control and neurodegenerative diseases, highlighting their potential roles in disease mechanisms and therapeutic targets [42]. The KEGG pathway analysis of Parkinson's disease highlighted multiple dysregulated biological mechanisms including mitochondrial dysfunction, oxidative stress,

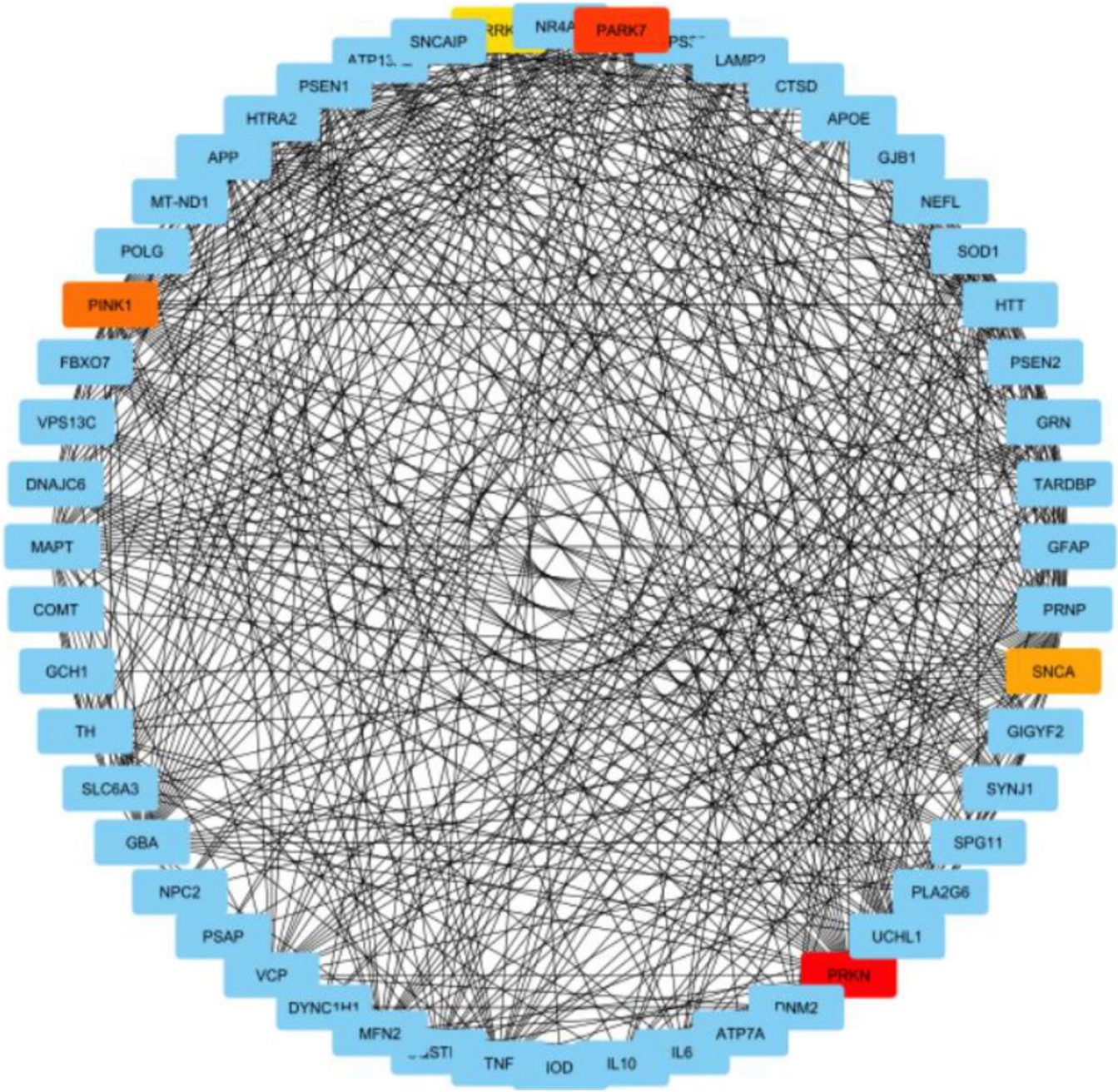

Fig. 2. Compound-target-anxiety network constructed by Cytoscape v_3.7.1.

impaired protein degradation and α-synuclein aggregation. Key genes such as SNCA, PARK2, PINK1, LRRK2 and UCHL1, all central to these pathways, were identified as crucial targets (Fig. 3).

Drug likeness: *In silico* Lipinski and ADMET analysis plays a pivotal role in the identification and development of drug candidates, as it ensures that compounds not only exhibit effective binding to their biological targets but also possess favourable pharmacokinetic profiles. The ADMET screening was conducted on the 130 compounds identified through GC-MS analysis, resulting in the selection of 4 compounds, which are detailed in Table-2. The data facilitated accurate classification of compounds based on their compliance with the defined parameter thresholds. The given parameters range are molecular mass: ≤ 500, high lipophilicity (Log P): ≤ 5, number of hydrogen bond donors: ≤ 5, number of hydrogen bond acceptors: ≤ 10, molar refractivity: 40-130, number of rotatable atoms: ≤ 9, topological polar surface area (TPSA (Å^2)): 20-130, solubility-log S (ESOL): -1 to -5, Absorption in gastric-intestine tract (GI absorption): high, blood brain barrier (BBB) permeant: yes, P-gp substrate: No, CYP1A2 inhibitor: No, CYP2C19 inhibitor: No, CYP2C9 inhibitor: No, CYP2D6 inhibitor: No, CYP3A4 inhibitor: No, Log Kp (skin permeation) cm/s: -1 to -6, bioavailability score: 0.55 [43]. Few of the compounds were segregated from other compounds in which nine compounds that include geraniol and its compounds were selectively collected for docking studies [44]. The selected 4 compounds showed good properties like solu-

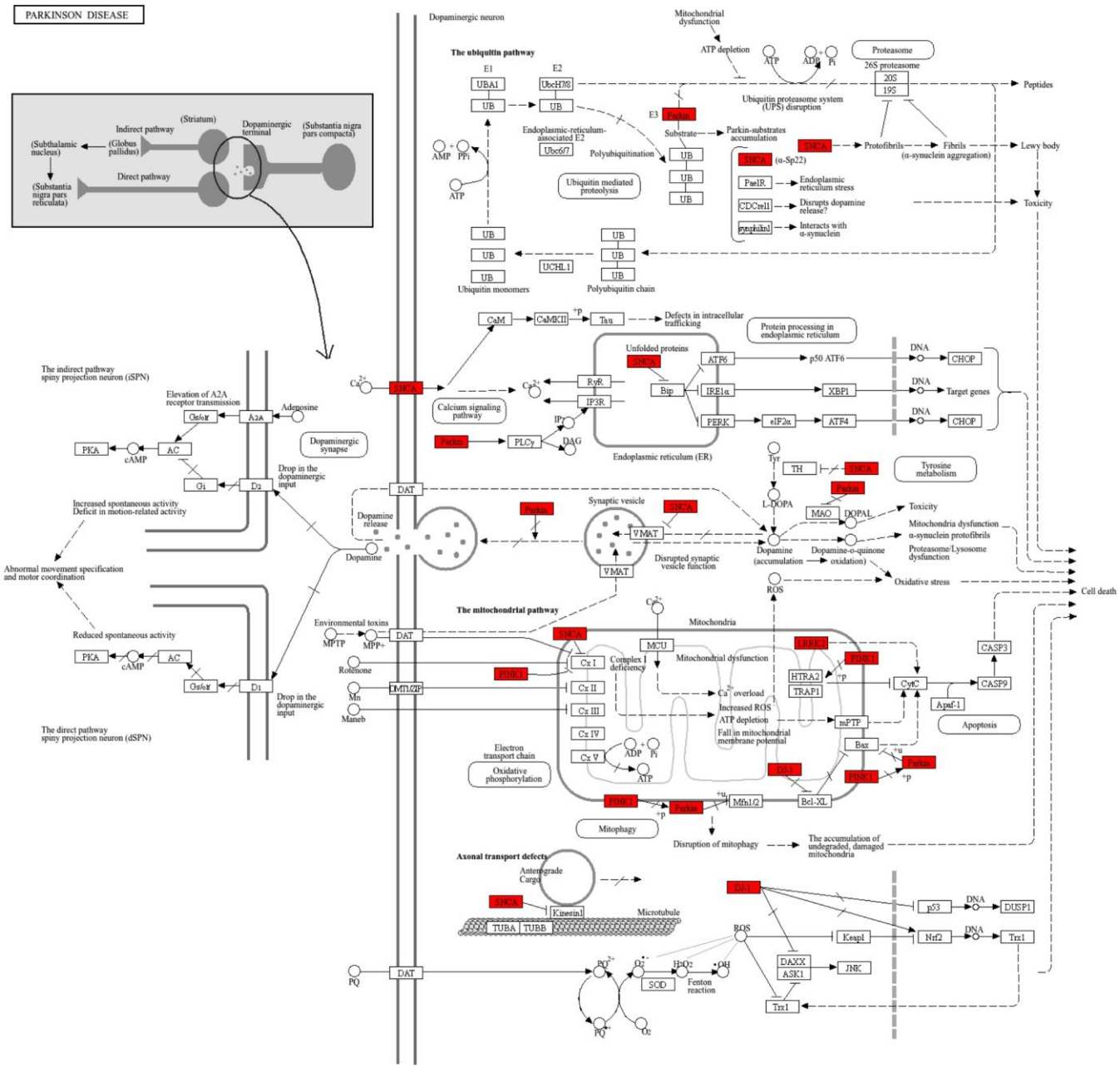

Fig. 3. KEGG pathway

bility, absorption in GI tract, crossing of BBB which can show the effect on central nervous system (CNS) and other pharmacokinetics properties as well.

Docking analysis: Before molecular docking, active compounds are evaluated using Lipinski's rule of five. Following this criterion, few compounds from *T. cordifolia* are found to meet Lipinski's rule of five and the ADME analysis. N,1-dimethyl-4-piperidinamine possessed the lowest binding energy of -7.32 kcal/mol, implying the highest binding affinity among the evaluated compounds. This indicates a significant potential for interaction with α-synuclein as a neuroprotective drug, as Levodopa, the reference drug for Parkinson's treatment, exhibited a binding energy of -5.98 kcal/mol, which is less favourable than that of N,1-dimethyl-4-piperidinamine [45]. Other compounds such as β-asarone (binding energy: -5.5 kcal/mol), 1,3,5-benzenetriol (binding energy: -4.68 kcal/mol) and 1,1-diethoxy-2-methyl- propane (binding energy: -4.31 kcal/mol) also showed moderate binding affinities and notable hydrogen bonding interactions with key residues like TYR168, TYR177, TRP159, GLU329 and LYS171 within the protein binding pocket (Table-3). The docking interactions were supported by hydrogen bonding, van der Waals forces and hydrophobic interactions, which contribute to complex stability are elucidated in Fig. 4 [46]. Studies evaluating the natural compounds using similar molecular docking approaches have reported comparable results [47].

Antioxidant activity: The *T. cordifolia* extract exhibited a significantly higher percentage of inhibition compared to Quercetin across all tested concentrations. At 100 µg/mL, *T. cordifolia* showed approximately 80% inhibition, while quer-

TABLE-2
ADME PROPERTIES FOR *Tinsopara caridifolia* EXTRACT

Compound name	m.f.	m.w.	No. of heavy atoms	No. of aromatic heavy atoms	No. of rotatable bonds	No. of H-bond acceptors	No. of H-bond donors	TPSA	ESOL Log S	GI absorption
Propane, 1,1-diethoxy-2-methyl-	$C_{12}H_{20}N_2O_3$	240.3	17	0	4	3	1	58.64	-1.72	High
Dimethyl(1-cyclopentylethoxy)silane	$C_{11}H_{13}NO_3$	207.23	15	6	6	3	1	55.4	-1.69	High
β-Asarone	$C_{12}H_{16}O_3$	208.25	15	6	4	3	0	27.69	-3.05	High
1,3,5-Benzenetriol	$C_6H_6O_3$	126.11	9	6	0	3	3	60.69	-1.22	High

Compound name	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)	Lipinski violation	Bioavail-ability score
Propane, 1,1-diethoxy-2-methyl-	Yes	Yes	No	No	No	No	No	-7.03	0	0.55
Dimethyl(1-cyclopentylethoxy)silane	No	No	No	No	No	No	No	-6.81	0	0.55
β-Asarone	Yes	No	Yes	Yes	No	No	No	-5.44	0	0.55
1,3,5-Benzenetriol	Yes	No	No	No	No	No	Yes	-6.96	0	0.55

TABLE-3
MOLECULAR DOCKING ANALYSIS OF FLAVONOID IN *Tinospora cordifolia* EXTRACT AGAINST -α-SYNUCLEIN

Compound	van der Waals interaction	Binding energy (Kcal/mol)	No. of H bonds	Hydrogen interaction	Total no. of residues
Propane, 1,1-diethoxy-2-methyl-	ILE 330, GLU 329, LYS 257, GLY 167, LYS 171	-7.32	2	PHE 170, ALA 169	ILE 330, GLU 329, LYS 257, GLY 167, LYS 171, PHE 170, ALA 169, PRO 332, PHE 259, TRP 159, TYR 177, TYR 168
1,3,5-Benzenetriol	TRP 63, PRO 155, ARG 345, MET 331	-4.68	5	ASP 66, ARG 67, GLU 45, GLU 154, TYR 156	TRP 63, PRO 155, ASP 66, ARG 67, GLU 45, GLU 154, TYR 156, ARG 345, MET 331
β-Asarone	LYS 171, TYR 168, TRP 159, PRO 332, GLU 329, TYR 177	-5.5	1	TYR 172	LYS 171, TYR 168, TRP 159, PRO 332, GLU 329, TYR 177 TYR 172, PHE 170
Levodopa	TRP 341, MET 331, LEU 236	-5.98	5	ARG 67, TRP 63, ASP 66, ALA 64, GLU 112	TRP 341, MET 331, LEU 236, ARG 67, TRP 63, ASP 66, ALA 64, TYR 156, TRP 231

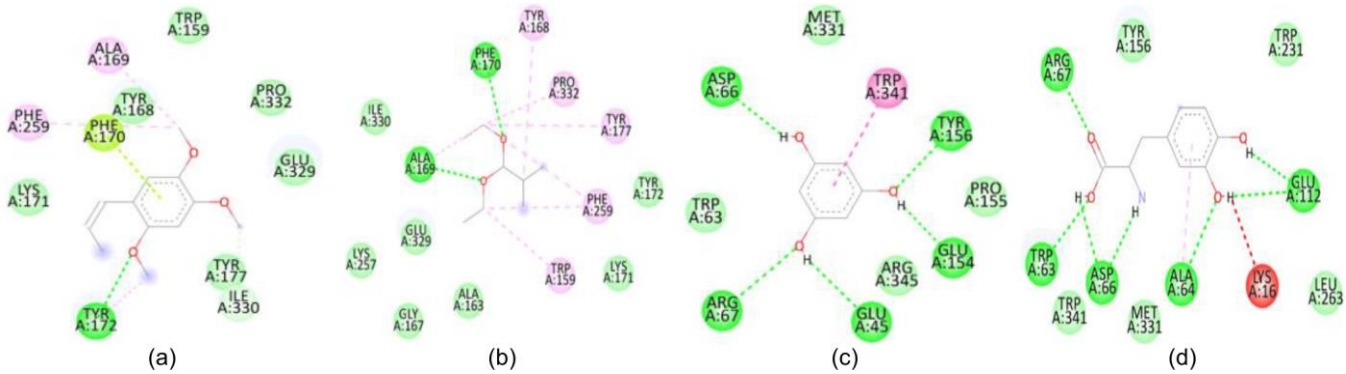

Fig. 4. Molecular intraction with natural *Tinospora cardifolia* plant flavonoid compounds for Parkinson's disease targeting α synuclein [PDB ID: 3Q27) 2D structure view during auto dock (a) β-asarone, (b) propane, 1,1-diethoxy-2-methyl-, (c) 1,3,5-benzenetriol and (d) control Levodopa

cetin showed only about 25%. As the concentration increased, both the extract and the standard showed a dose-dependent increase in radical scavenging activity. At 500 µg/mL, *T. cordifolia* achieved inhibition close to 85%, whereas quercetin reached about 75% (Fig. 5). These findings suggest that the *T. cordifolia* extract possesses potent antioxidant activity, potentially superior to that of quercetin. Thus, *T. cordifolia* extract could be a promising natural antioxidant with potential applications in oxidative stress-related disorders.

The FRAP (ferric reducing antioxidant power) assay evaluated the antioxidant potential of the *T. cordifolia* extract in comparison to the standard antioxidant, gallic acid, across

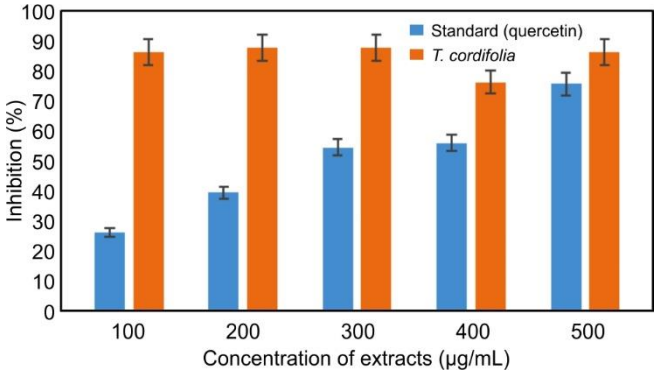

Fig. 5. DPPH assay activity with standard for *T. cordifolia* extract

a concentration range of 100-500 µg/mL. From Fig. 6, it is evident that gallic acid exhibited a concentration-dependent increase in antioxidant activity, with inhibition percentages rising from approximately 30% at 100 µg/mL to around 70% at 500 µg/mL. In contrast, the *T. cordifolia* extract showed relatively low and nearly constant reducing power across all concentrations, with values ranging between ~20-25%. This indicates that while the *T. cordifolia* extract does exhibit some reducing ability, it is significantly lower than the standard. Unlike the DPPH assay where *T. cordifolia* performed robustly, the FRAP results suggest that *T. cordifolia* may not be as effective in reducing ferric ions, implying a selective antioxidant mechanism [48].

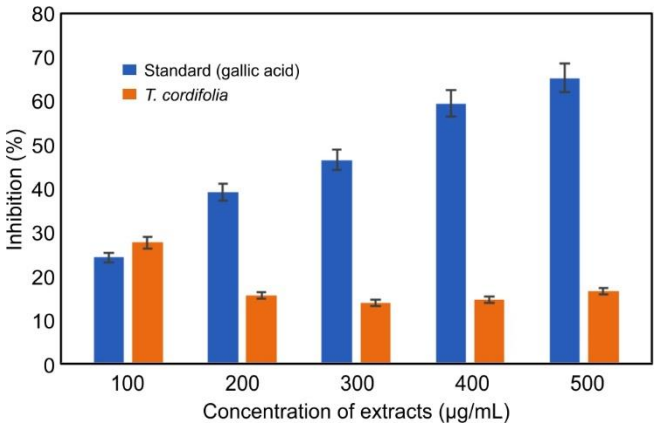

Fig. 6. FRAP assay activity with standard for *T. cordifolia* extract

Conclusion

This study highlights the neuroprotective and therapeutic potential of flavonoid compounds derived from the ethanol extract of *T. cordifolia* in Parkinson's disease, particularly by targeting α-synuclein (SNCA), a central protein in Parkinson's disease pathogenesis. Through comprehensive GC-MS analysis, multiple bioactive phytoconstituents were identified in ethanol extract of *T. cordifolia*, several of which demonstrated significant antioxidant properties and drug-likeness based on Lipinski's rule and ADMET screening. Compound, N,1-dimethyl-4-piperidinamine, showed the strongest binding affinity (-7.32 kcal/mol) against SNCA in molecular docking studies, surpassing the standard levodopa, which exhibited a lower binding energy (-5.98 kcal/mol). In addition to molecular docking, the antioxidant assays confirmed the biological efficacy of *T. cordifolia* extract. In the DPPH assay, *T. cordifolia* extract demonstrated superior radical scavenging activity, achieving over 85% inhibition at 500 µg/mL, significantly higher than quercetin, the standard. However, the FRAP assay results indicated a more selective antioxidant mechanism, as *T. cordifolia* exhibited lower ferric reducing capacity than gallic acid, suggesting that *T. cordifolia* primarily acts *via* free radical neutralization rather than ferric ion reduction. Network pharmacology analyses further supported the therapeutic promise of *T. cordifolia* due of the presence of flavonoids. Key hub genes such as SNCA, LRRK2, PARK7 and PRKN, were mapped in the protein-protein interaction network and KEGG pathway enrichment confirmed involvement in mitophagy, oxidative stress and synaptic signalling central elements in Parkinson's disease pathology. Further *in vivo* and clinical studies are warranted to validate these bioactive compounds for the therapeutic applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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