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In silico studies and antioxidant activity of *Pongamia pinnata* against the potential targets of Parkinson's disease

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A degenerative neurological condition of the brain, Parkinson's disease (PD) occurs when dopamine producing cells become less. *Pongamia pinnata* seed extract has demonstrated its ability to mitigate neuronal damage, reduce oxidative stress, and enhance neuronal survival in various experimental models of neurodegenerative diseases. Hence, the primary goal of this research is to investigate the prospective abilities of flavonoid compounds isolated from *Pongamia pinnata*. The quantitative analysis revealed that phytochemicals extracted from ethanol showed greater flavonoid content than other solvent. Gas chromatography - Mass Spectrometry (GC-MS) investigation was also conducted to determine the number of flavonoid compounds contained in the crude extracts of the plant. Additionally, *Pongamia pinnata* seed extract has been demonstrated to regulate critical molecular pathways that are affected by neurodegeneration, such as the regulation of apoptosis, inflammation, and the oxidative stress response. Flavonoids from *Pongamia pinnata* were screened and docked with nine distinct proteins using AutoDock 4.2. Flavonoid compound 2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone emerged as the top binding compounds from *Pongamia pinnata*, showing significant binding across multiple genes in the neurodegenerative pathway. DPPH and FRAP antioxidant assay were performed for the plant extract and the inhibition activity was found to be 54.28% and 54.21%, respectively. These findings underscore the therapeutic potential of *Pongamia pinnata* seed extract as a promising candidate for the prevention and treatment of neurodegenerative disorders.

Keywords: Blood brain barrier (BBB), Docking analysis, Flavonoids, Inhibition activity, Neurodegenerative diseases

Neurological disorders include a wide range of long-term illnesses with very complicated causes[1]. More than 10 million people around the world have neurological illnesses every year, and that number is likely to go up in the years to come. The brain's ability to work decreases with age because of neurodegenerative processes. This has led to the discovery of cellular and molecular targets that improve brain performance. Disorders of the neuronal mechanisms can produce a broad spectrum of symptoms and impact different parts of the nervous system. Epilepsy is a condition that impacts the electrical activity of the brain and is distinguished by recurrent convulsions. Alzheimer's disease is a neurodegenerative disorder that progresses over time and results in cognitive decline, memory loss, and behavioural alterations[2,3]. Parkinson's Disease is a movement disorder that results in tremors, rigidity, and challenges with balance and coordination because of brain cells that generate reduced dopamine. The autoimmune disease - Multiple sclerosis (MS) causes breaks in connection between the brain as well as the rest of the physique by means of an unintentional attack by the immune system against the protective covering of nerve filaments[4,5]. Amyotrophic Lateral Sclerosis (ALS) is a persistent neurodegenerative condition that results in deteriorating muscular movement, paralysis, and, finally respiratory failure referred to as Lou Gehrig's disease. Stroke is a disorder where the blood flow to the brain is disturbed leading to damage of brain cells with a variety of neurological impairments. Recurrent severe migraines that are frequently accompanied by vertigo, vomiting, and sensitivity to light and sound is referred to as migraine[6]. The developmental disease Autism Spectrum Disorder (ASD) affects interactions with others, conduct, and communication. An inherited disorder that causes brain nerve cells to gradually degenerate, which in turn leads to cognitive decline and motor dysfunction is referred to as Huntington's Disease. Cerebral palsy is a collection of conditions that frequently result from brain injury sustained

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during pregnancy, childbirth, or the immediate postpartum period. These conditions affect muscle coordination, posture, and movement[7].

A neurodegenerative condition that impacts the central nervous system is Parkinson's disease, specifically in region of the brain that modulates motor control. It is a long-term condition. Parkinson's disease, which was initially characterised by the British physician James Parkinson, is characterised by the gradual degradation of nerve cells in the nerve root, an area that is crucial for the regulation of muscular movement and coordination. Parkinson's disease patients frequently experience bradykinesia (slowness of movement), rigidity, and tremors. Furthermore, the clinical presentation may be further confounded by the presence of non-motor actions including autonomic dysfunction, emotional problems, and cognitive decline[8,9]. The exact genesis of Parkinson's disease remains anonymous, nonetheless, it is thought that a combination of environmental and genetic factors has a role in its genesis. Unfortunately, there is no fix for Parkinson's disease yet. However, there are a number of treatments that can help people with the disease live better and feel better. These include medication, physical therapy, and in some cases, surgery.

Researchers are examining the complicated underpinnings of Parkinson's disease while pursuing advancements in neurology and medical science to create novel therapeutics and enhance comprehension of this delicate neurological ailment. This introduction marks the beginning of an exploration into the complex nature of Parkinson's disease, encompassing its effects, problems, and the advancing realm of research and treatment alternatives[10]. *Pongamia pinnata*, which is additionally known as the Indian beech or Pongam tree, is a plant that can be used for numerous medicinal purposes. It is mainly recognised for the uses it has in traditional medicine, especially in Ayurveda. But investigation on its effect in treating neurological disorders is still going on, but it is very limited. Many investigations have shown that the antioxidant and anti-inflammatory chemicals in *Pongamia pinnata* may help protect brain neurons. Protecting neurons from damage is an important part of stopping or slowing the development of neurological disorders. It does this by keeping neurons' structure and function. Researchers investigated whether Karanjin, which is one of the main parts of *Pongamia pinnata*, could be useful for health. Different research projects have shown that Karanjin may help protect neurons and could be useful in the treatment of Alzheimer's, Parkinson's, and stroke. It has anti-oxidant and anti-inflammatory properties that helps lower inflammation in the brain and damage to neurones[11,12]. This research work focuses on exploring the antioxidant potential and *In silico* analysis of compounds identified in *Pongamia pinnata* against various targets of Parkinson's disease.

Materials and Methods

Sample collection and preparation of extracts

Pongamia pinnata seedswere collected from Krishnagiri district Kaveripattinam, Tamil Nadu, India (between 11°12'N to 12°49'N Latitude and 77°27'E to 78°38'E Longitude). The sample was identified and authenticated by Dr.K. Rajagopal, Assistant Professor, Department of Botany, Ramakrishna Mission Vivekananda College, Chennai, India. The seeds were shade dried and grinded into a coarse powder. 10 g of sample was weighed and separately extracted with 100 mL of 3 different solvent (ethanol, ethyl acetate, water) using ultrasonication method with 30 minutes time at room temperature (27- 32°C). The extract was then separated from solid debris by filtration and solvent was dried into powder by evaporation at room temperature.

Phytochemical screening

The seed extracts of *Pongamia pinnata* using different solvents (ethanol, ethyl acetate and water) were subjected to qualitative analysis of phytochemicals such as alkaloids, phenols, flavonoids, steroids, terpenoids, tannin, proteins, amino acids and carbohydrates[13].

Quantitative analysis of flavonoid

The Aluminium chloride ($AlCl_3$) complex formation assay was used to estimate the total flavonoid content. With quercetin as a standard, flavonoid content was calculated as the quercetin equivalent. Different concentrations of the standard quercetin mixture made in ethanol (0.1, 0.5, 1.0, 2.5, and 5 mg/mL) and addition of 500 µL of pure water. 100 µL of 5% sodium nitrate was then added, and the mixture was incubated for six minutes. After five min, 150 µL of a 10% $AlCl_3$ solution was then added to the mixture, and then 200 µL of a 1M sodium hydroxide solution was added. Optical Density values were measured at 510 nm and the readings were recorded. The measurements were taken in triplicates and the concentration (x) was determined from standard curve

of quercetin where y is the concentration and x is the standard deviation[14].

GC-MS analysis

The GC-MS analysisof sample was given at SRM University, Kattankulathur, Chennai, Tamil Nadu. Gas chromatograph (Shimadzu, QP2010 plus) was used to analyse the ethanol extracts of the *Pongamia pinnata*. The oven was set to 50°C at first, and the injection temperature was set to 250°C at a rate of 10°C/min. Helium was used as the carrier gas, and an average flow rate of 1mL/min was used. The data collection was administered using parted sampling method with the split ratio index of 40.0 and carrier gas saver split ratio of 5.0. In comparison to the retention periods of a series, the duration of retention indicators (RI) of the phytochemicals was computed. Hence, the identity of every component was verified by correlating the persistence rate with those of validated substances or data from reputable sources[15].

Selection of protein

A broad assessment of the neurodegenerative disease pathology led to the analysis of several kinds of genes involved in crossing of the blood brain barrier along the sides of endothelial cells, leading to various neurodegenerative disorders. These genes were also mutated along their route to bypass the BBB and cause neurodegenerative disorders[16]. Thus, nine genes were screened using various literature research, and their protein ID was chosen based on attributes such as x-ray diffraction, molecular weight, and the number of amino acids in their chains. All protein IDs have been retrieved from Protein Data Bank (PDB).

Protein structure modelling by *in silico activity*: Swiss Model

The unknown structure of the proteins coiled-coil-helix-coiled-coil-helix domain_2 (CHCHD2) and Williams-Beuren syndrome chromosome region 17 (WBSCR17) was predicted by Swiss Model. a template search was conducted using the SWISS-MODEL template library (SMTL) by employing two database search techniques such as HHblits, which enhances sensitivity for detecting remote homology, and BLAST, known for its speed and accuracy with closely related templates. Following the template identification, a 3D protein structure was generated autonomously for each chosen motif. The positions of the atoms that are the same in both the target and template sequences were used, and any changes (insertions or deletions) found in the alignment were accounted for loop modelling. Additionally, building the side chains of the non-conserved amino acids created a complete model of the protein using the ProMod3 modelling tool. Finally, to validate the structure, the Ramachandran plot was utilized to visualize the dihedral angles of amino acid residues, while the f pocket tool was employed to determine the active site[17,18].

Molecular docking analysis

The compounds identified using GC-MS analysis of the ethanol extract of the *Pongamia pinnata* were studied for its drug likeness using Lipinski's rule of five. Lipinski's rule of 5 parameters such as molecular weight, log P, number of hydrogen bond donors, number of hydrogen bond acceptors and molecular refractivity were taken from the PubChem database for the compounds. Docking studies were performed using an automated bioinformatic docking tool, AutoDock 4.2. The structures were converted in .pdbqt format and interactions were predicted using Lamarckian Genetic algorithm. It comprises of two approaches which involves efficient investigation of torsional flexibility and grid-based energy assessment[19,20]. Discovery Studio Visualizer was used for visualizing the molecular structures, sequences, and the interaction patterns.

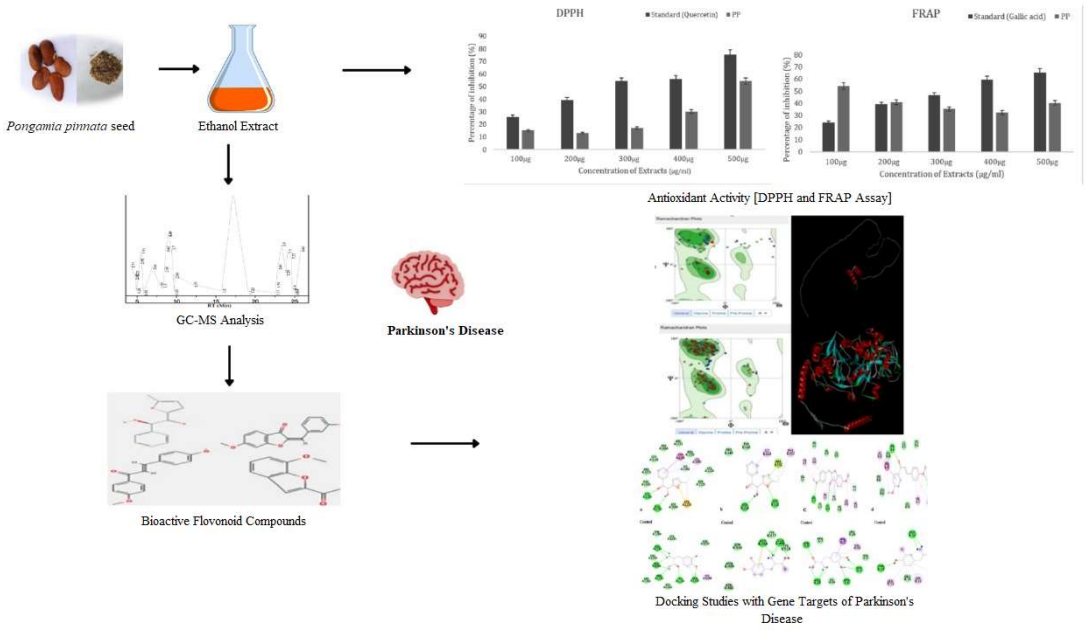
DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The antioxidant activity of the samples was tested by the DPPH method, Varying concentrations of plant extracts (100-500 µg/mL) were added to 2 mL of DPPH solution (2 mg/mL in methanol). The blank was made using a diluted DPPH solution in methanol. Quercetin was used as positive control (100 µg/mL). This reaction mixture was maintained in darkness at room temperature for 30 minutes, and the absorbance value was recorded at 517 nm[21,22].

$$\text{Percentage of inhibition} = (\text{Abs control} - \text{Abs sample}) \text{ X } 100 / \text{ Abs control}$$

Ferric reducing antioxidant power (FRAP) assay

The FRAP (Fluorescence Recovery after Photobleaching) reagent was mixed with 1mL of different concentrations of sample (100-500 µg/mL). The mixture was kept at incubation for 30mins at 37°C. The resultant mixture turned into blue colour complex where the ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was reduced to ferrous (Fe^{2+}) form. The absorbance at 593 nm was assessed relative to a



Graphical abstract

blank (FRAP reagent combined with distilled water). The experiments were conducted in triplicate[23].

Percentage of inhibition = (Abs control − Abs sample) X 100/ Abs control

Result and Discussion

Analysis of phytochemicals in *Pongamia pinnata* revealed the presence of different bioactive substances. Table 1 depicts the presence and lack of these phytochemicals in three distinct *Pongamia pinnata* extracts. Alkaloids (ethyl acetate, ethanol, water), flavonoids (ethanol, ethyl acetate, aqueous), and terpenoids (ethyl acetate) were detected in the preliminary test for phytochemical analysis. Alkaloids are important for pharmacological actions for anti-malarial, anti-cancer, and anti-hyperglycemic effects[24].It is well known that terpenoids have antibacterial properties. Antioxidants included in flavonoids may aid in shielding body cells from damaging free radicals.

Quantitative analysis of flavonoids

The quantity of flavonoids in all three freeze-dried filtrate samples (ethanol, ethyl acetate, aqueous) was assessed using the aluminium chloride colorimetric assay, employing quercetin as a standard. A standard calibration curve for quercetin was generated based on the absorbance values of different quercetin concentration[25]. This procedure was repeated for the freeze-dried samples of *Pongamia pinnata* extract and the total flavonoid concentration was quantified in micrograms of quercetin equivalents per milligram as depicted in (Table 2 and Fig. 1). Each step was performed in triplicate, and the concentration (x) was determined by substituting the absorbance value of the sample (y) into the standard curve equation1, where y represents the measured absorbance and x denotes the unknown concentration.

Table 1 — Phytochemical analysis of *P. pinnata* seed extract

S. No	Test	Ethanol	Ethyl acetate	Aqueous
1	Alkaloid	Present	Present	Present
2	Saponins	Absent	Absent	Absent
3	Protein and amino acids	Absent	Absent	Absent
4	Ninhydrin test	Absent	Absent	Absent
5	Carbohydrates	Absent	Absent	Absent
6	Phenol	Absent	Absent	Absent
7	Flavonoids	Present	Present	Present
8	Tannins	Absent	Absent	Absent
9	Steroids	Absent	Absent	Absent
10	Terpenoids	Absent	Present	Absent

Table 2 — Total flavonoid content in *Pongamia pinnata*

S. No	Solvent	Total Flavonoid Content (TFC) mg/g
1	Ethanol	9.6±1.2
2	Ethyl acetate	5.92±1.4
3	Aqueous	3.07±1.9

Gas chromatography- Mass spectrometry analysis

The GC-MS analysis of *P.pinanata* seed ethanol extract contained the major bioactive components such as Dimethylsulfoxoniumformylmethylide, Ethanethiol, 2-(dimethylamino)-, N-2- chloroethyl-n,n-dimethyl ammonium chloride, Silanol, trimethyl-, formate, 2-thiophenepropanamine, n,n-dimethyl-, 2,2-dimethyl-1,3-butanediol, 2-pyrrolidinone, 1-methyl-, 2-cyclopenten-1-one, 2-hydroxy-3-methyl- *etc*. as given in (Table 3). The retention time of the identified compounds are shown in (Fig. 2). These bioactive compounds consist of amine groups, alkyl groups, flavonoids, steroids, fatty acids, ether and alcohol groups[26]. The biological activity of the identified compounds are given in (Table 2).

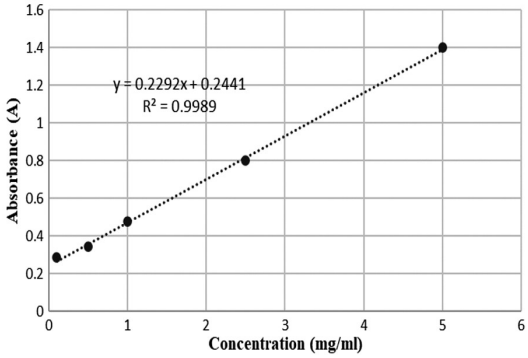


Fig. 1 — Standard Curve of Quercetin (TFC)

Ligand selection

The Lipinski rule of five was applied to analyse the active compounds. The molecular mass of each compound must be less than 500 Dalton for the five principles of drug likeness to be applicable. As per Lipinski's "rule of five", five key physiochemical parameters (molecular weight, lipophilicity, polar

Table 3 — The GC-MS characterization with bioactive components with biological activity

S. No	Compound name	Activity	RT
1	Dimethylsulfoxoniumformylmethylide	Antibacterial, Antidiabetic, Anti-oxidant	4.188
2	Ethanethiol, 2-(dimethylamino)-	Anticancer activity	4.692
3	N-2- chloroethyl-n, n-dimethyl ammonium chloride	Ant-estrogenic activity, antibacterial activity	4.773
4	Silanol, trimethyl-, formate	Antimicrobial agent	4.913
5	2-thiophenepropanamine, n, n-dimethyl-	Anti-depressant agent, Anxiolytic agent	5.339
6	2,2-dimethyl-1,3-butanediol	Anti-inflammatory	5.527
7	2-pyrrolidinone, 1-methyl-	Anti-bacterial activity	5.805
8	2-cyclopenten-1-one, 2-hydroxy-3-methyl-	Sweetener, Anti-bacterial activity	6.949
9	6-methyl-hept-2-en-4-ol	Anti-bacterial and Anti -oxidant activity	7.857
10	1,2,3-cyclohexanetriol-o, o', o"-d3	Anti-cancer activity, vitamin D supplement	8.291
11	2-isopropyloxan-4-ol	Anti-oxidant and Anti-bacterial	8.491
12	3-hydroxy-4,4-dimethyldihydro-2(3h)-furanone	Anticancer, Anti-depressant	8.652
13	Butane, 1,1'-oxybis (3-methyl-	Antioxidant, Anti-cholinesterase, Antibacterial activity	8.923
14	4h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Anti-oxidant	9.346
15	2-hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone	Anti-oxidant, Anti-cancer activity	9.714
16	9-phosphabicyclo (3.3.1) nonane	Anti- inflammatory	9.807
17	Stevioside	Sweetener	12.162
18	3(2h)-benzofuranone, 6-methoxy-2(3-methoxyphenyl) methylene)-, (e)-	MAO inhibitor	25.765
19	Heptasiloxane, hexadecamethyl	Anti-inflammatory activity	25.07
20	Methanone, phenyl(3-tridecyloxiranyl)-	Anti-depressant, Anti-inflammatory, Antimicrobial activity	24.979
21	4,4′-dimethoxychalcone	Anti-oxidant activity, Anti-tumour, Anti-bacterial activity	24.884
22	thiourea, n-(3-(methylthio)propyl)-	Neuroprotective	24.676
23	ketone, 7-methoxy-2-benzofuranyl methyl	Anti-oxidant, bacterial, tumour	15.746
24	E, E-3,13-octadecadien-1-ol	Anti-profilative, Anti-oxidant, Anti- inflammatory	24.106
25	N-(bromomethyl)phthalimide	Anti-analgesic, Anti-inflammatory	23.865
26	Z-11-pentadecenol	Anti-bacterial and Anti-oxidant activity	23.358
27	Palmitoyl chloride	Anti-microbial and Anti-oxidant activity	22.538
28	S-(2-(n, n-dimethylamino)ethyl)n,n-dimethylcarbamoylthiocarbohydroximate	Atherosclerosis, Anti-oxidant	22.86
29	Hexasiloxane, tetradecamethyl-	Anti-cancer, Anti-oxidant, cardiac disease	22.64
30	Acetamide, 2-(diethylamino)-n-(2,6-dimethylphenyl)-	Anti-arrhymatic activity, Anti-epileptic	19.356
31	4-phenylpent-3-en-2-one	Neuroprotective activity	19.100
32	Mome inositol	Panic disorder, Anti-depression, attention deficit	17.18

surface area, hydrogen bonding, and charge)[27]. The ligand satisfying Lipinski's rule of five are shown in (Table 4).

Selection of target genes for *In silico* analyis

After a thorough analysis of the pathophysiology of neurodegenerative diseases, it was determined that several different gene types were implicated in the BBB crossing process along the sides of endothelial cells, which results in a variety of neurodegenerative diseases. LRRK2 (Leucine-rich repeat kinase 2provides instructions for synthesising a protein that regulates multiple physiological functions, including neuronal function. Mutations in this gene can lead to dysfunctional protein activity, which may facilitate the emergence of Parkinson's disease[28]. An enhanced prevalence of Parkinson's disease has been associated to mutations in the PARK7 gene. DJ-1, a protein that generates by the PARK7 gene, has the ability to protect against toxicity caused by free radicals and migrate to the outer mitochondrial membrane in an instance of oxidative stress. Variations in the PINK1 gene have been associated to a rare type of autosomal recessive Parkinson's disease. The PINK1 protein regulates metabolic function and quality control[29]. Variations in this gene may impair functioning of the mitochondria resulting in the proliferation of defective mitochondria leading to cellular apoptosis. Alpha-synuclein is a protein found throughout the brain and is a primary element of Lewy bodies, which are protein clumps located in brain of persons affected with Parkinson's disease. Mutations in the SNCA gene mostly encrypted for alpha-synuclein, can result in the inappropriate accumulation and aggregation of this protein, which is thought to contribute to the degeneration of dopaminergic neurons in Parkinson's disease[30]. Through an extensive literature review, nine genes crossing the blood-brain barrier in neurodegenerative disorders were identified, and their corresponding protein IDs were selected from the RCSB PDB. The list of genes selected are shown in (Table 5).

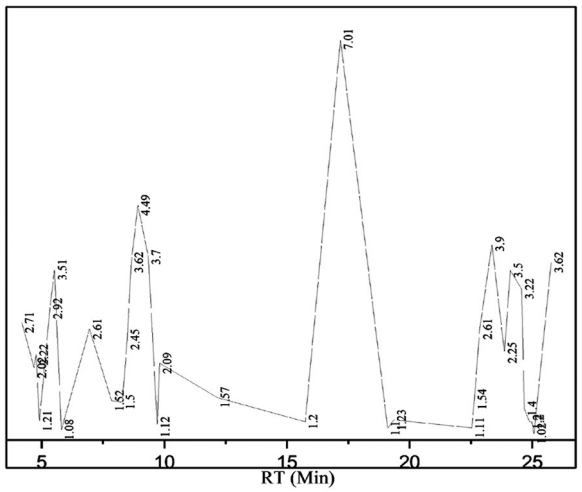


Fig. 2 — The GC-MS chromatogram graph of *Pongamia pinnata* ethanol extract

Homologous modelling of protein- Swiss model

Two of the nine proteins that had previously been screened, CHCHD2 and WBSCR17, do not have protein ID in PDB. As a result, protein structure modelling using SWISS MODEL was performed. Creating three-dimensional protein framework prototypes using empirically determined structures from members of the

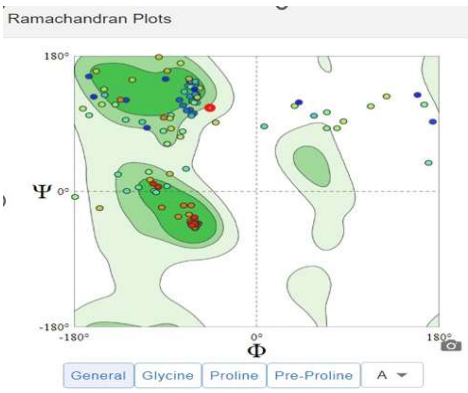
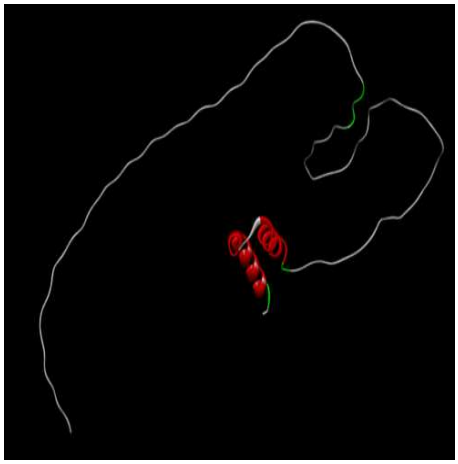
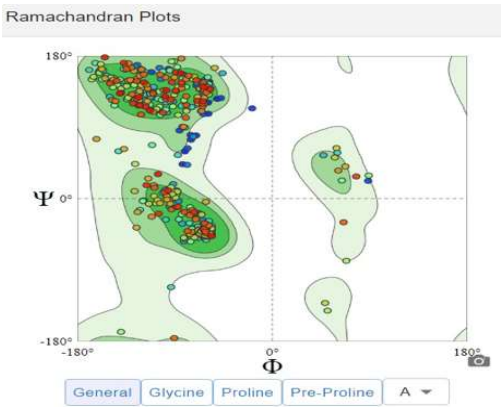
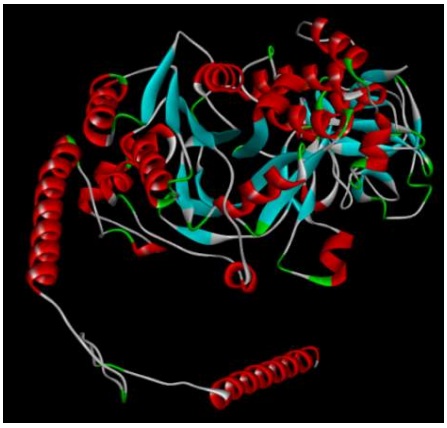
Table 5 — List of genes selected for docking studies

S. No	Gene name	PDB ID
1	Leucine rich repeat kinase 2 (LRRK2)	5VBO
2	Parkinsonism associated deglycase (PARK7)	3EZG
3	PTEN induced kinase 1 (PINK1)	6GLC
4	Parkin RBR E3 ubiquitin protein ligase (PRKN)	4I1F
5	Prosaposin (PSAP)	3BQP
6	Synaptic vesicle glycoprotein 2C (SV2C)	6ES1
7	Synuclein alpha (SNCA)	3Q27
8	Coiled-coil-helix-coiled-coil-helix domain 2 (CHCHD2)	Homologous modelling
9	Williams-Beuren syndrome chromosome region 17 (WBSCR17)	Homologous modelling

Table 4 —Lipinski rule 5 satisfied components

S. No	Phytochemical Compound	Molecular weight (<500)	Log P (<5)	H-bond Donor (<5)	H-bond acceptor (<100)	Molar refractivity (<130)
1	2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone	216	2.504	1	3	59.17
2	3(2H)-Benzofuranone,6-methoxy-2-(4-methoxyphenyl) methylene)-(E)-	282	3.31	0	4	78.92
3	4,4′-Dimethoxychalcone	268	3.599	0	3	79.35
4	Ketone,7-methoxy-2-benzofuranyl methyl	190	2.64	0	3	52.77

Table 6 — Homologous modelling proteins CHCHCD2 AND WBSCR17

Gene name	Ramachandran plot	3D structure of the protein
Coiled-coil-helix-coiled-coil-helix domain_2 (CHCHD2)		
Williams-Beuren syndrome chromosome region 17 (WBSCR17)		

same family as a guide is the aim of homology modelling.Unique protein frameworks are searched to find ideal templates for the target proteins[31]. The target protein is modelled in three dimensions, and Ramachandran plots are examined (Table 6).

Molecular docking analysis

Molecular docking analyses of flavonoid compounds was conducted to assess the binding energy of each compound and their potential as therapeutic agents against neurodegenerative diseases.The compounds of *Pongamia pinnata* docked using nine different genes and the binding energy obtainedare displayed in the (Table 7). Among all the compounds, 3(2H)-Benzofuranone,6-methoxy-2-((4-methoxyphenyl) methylene)-(E)-flavonoid compound from *Pongamia pinnata* extract with biological activity against MAOIs impede the function of monoamine oxidase A (MAO-A) and/or monoamine oxidase B (MAO-B) enzymes[32]. They stop the breakdown of tyramine in the body. This prevents the production of neurotoxic side products that can cause neuronal damage.4,4′-dimethoxychalcone and ketone,7-methoxy-2-benzofuranyl methyl controls and inhibit the oxidative stress level.2-hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone is a flavonoid compound with anti-depressant activity showed best binding energy when compared to other plant source compounds in PINK1 gene[33].The docking interactions of 3-(2H)-Benzofuranone, 6-methoxy-2((4-methoxyphenyl)methylene)- and 4,4′-Dimethoxychalnone has good binding energy with a greater number of hydrogen bonds (Fig. 3c & 3d) Mutations in the PINK1 gene either change or remove the kinase domain, therefore compromising protein activity. At least one mutation alters the mitochondrial-targeting motif and could interfere with protein delivery to mitochondria. Particularly in stressed-out cells, mitochondria may fail with lowered or missing PTEN driven putative kinase 1 activity[34]. Lack of energy for basic functions might cause cells to die. The mechanism

Table 7 — Binding energy of various flavonoid compounds for *Pongamia pinnata*

Gene	Ligand	Vanderwal's interaction	Binding energy	No. of hydrogen bonds	Hydrogen interactions	Total no of residues
6ES1_A (Fig.3a)	2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone	ARG 1232, TRP 1014, GLY 1102, ARG 1013, PHE 1100, ASP 1099, SER 1225, LYS 1224, LYS 1098, ASN 1233	-6.98	2	LYS 1234, GLU 1283	ARG 1232, TRP 1014, GLY 1102, ARG 1013, PHE 1100, LYS 1234, GLU 1283, ASP 1099, CYS 1235, SER 1225, LYS 1224, LEU 1097, ASN 1233, TRP 1101, LYS 1098
	3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl) methylene]-, (E)-	ALA 1049, SER 1050, GLU 969, ASN 970 GLN 992, ASN 990, SER 972, ASN 1051, ASN 930	-5.45	Nil	Nil	ALA 1049, SER 1050, GLU 969, ASN 970, HIS 1048, GLN 992, ASN 990, SER 972, ASN 1051, ASN 930, VAL 933
	4,4′-Dimethoxychalcone	GLN 1091, GLN 913, SER 1092, LYS 1092, LYS 1098, ASN 1233, GLU 1283, TYR 1088, GLY 1102	-6.46	2	ARG 1232, LYS 1070	GLN 1091, GLN 913, SER 1092, LYS 1092, LYS 1098, ASN 1233, GLU 1283, TYR 1088, GLY 1102, TRP 1014, TRP 946, CYS 1235, LYS 1234, ARG 1232, LYS 1070
	Ketone,7-methoxy-2-benzofuranyl methyl	LYS 1234, ASN 1233, LEU 1097, ILE 1096, SER 1094, SER 1225, TYR 1088, SER 1092	-5.3	2	LYS 1098, ARG 1232	LYS 1234, ASN 1233, LEU 1097, ILE 1096, SER 1094, SER 1225, TYR 1088, SER 1092, CYS 1235, LYS 1098, ARG 1232
	levodopa	PHE 1284, CYS 1235, ASN 1233, GLY 1102, TRP 1101, PHE 1100	-4.76	2	GLU 1283, ASP 1099, ARG101, LYS 1234, ARG 1232	LYS 1098, GLU 1283, ASP 1099, ARG 1013, LYS 1234, ARG 1232, PHE 1284, CYS 1235, ASN 1233, GLY 1102, TRP 1101, PHE 1100
6ES1_B (Fig. 3b)	2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone	PRO 549, TYR 550	-7.26	2	ASP 554, SER 555	ILE 553, PHE 557, PHE 552, TYR 550, PRO 549, ASP 554, SER 555
	3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl) methylene]-, (E)-	ASP 554, ILE 553, PHE 552, GLU 548, PHE 547, ASN 565	-6.42	1	SER 555	ASP 554, ILE 553, PHE 552, GLU 548, PHE 547, ASN 565, PRO 549, PHE 562, PHE 557, SER 555
	4, 4′-Dimethoxychalcone	ASN 559, LYS 558, PHE 557, PHE 552, PHE 547, GLU 548, ASN 565	-6.42	1	CYS 560	ASN 559, LYS 558, PHE 557, PHE 552, PHE 547, GLU 548, ASN 565, PHE 562, PRO 549, CYS 560
	Ketone,7-methoxy-2-benzofuranyl methyl	ASN 529, TYR 550, VAL 528, SER 527, ASP 546	-4.97	2	THR 530, LYS 551	ASN 529, TYR 550, VAL 528, SER 527, ASP 546, TYR 531, THR 530, LYS 551
	Levodopa	SER 519, ASN 499, ASN 480, LYS 477, LYS 518	-4.35	2	ASP 498, GLU 496	SER 519, ASN 499, ASN 480, LYS 477, LYS 518, ASP 498, GLU 496

(*Contd.*)

Table 7 — Binding energy of various flavonoid compounds for *Pongamia pinnata* (*Contd.*)

Gene	Ligand	Vanderwal's interaction	Binding energy	No. of hydrogen bonds	Hydrogen interactions	Total no of residues
6GLC_A (Fig. 3c)	2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone	ARG 42, PHE 7, LEU 41, PHE 4, LEU 61, LEU 50, PHE 45, PHE 4	-7.92	2	ILE 44, ARG 6	ARG 42, PHE 7, LEU 41, PHE 4, LEU 61, LEU 50, PHE 45, PHE 4, VAL 3, VAL 43, VAL 5, LYS 27, VAL 30, LEU 26, ILE 44, ARG 6
	3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl) methylene]-, (E)-	ARG 6, VAL 30, LEU 26, ASN 8, LYS 27, VAL 43, PHE 45, PHE 4	-7.43	2	ILE 44, ARG 42	ARG 6, VAL 30, LEU 26, ASN 8, LYS 27, VAL 43, PHE 45, PHE 4, PHE 7, LEU 61, LEU 41, VAL 5, VAL 3, ILE 44, ARG 42
	4,4′-Dimethoxychalcone	LYS 27, LEU 26, PHE 4, ARG 6, ASN 8, ARG 42	-6.96	Nil	Nil	LYS 27, LEU 26, PHE 4, ARG 6, ASN 8, ARG 42, LEU 41, VAL 30, PHE 7, VAL 43, VAL 5, VAL 3, PHE 45, LEU 61
	Ketone,7-methoxy-2-benzofuranyl methyl	ARG 42, PHE 7, ARG 6, ILE 44	-6.21	1	ASN 8	VAL 30, VAL 43, VAL 5, LEU 26, LYS 27, LEU 41, PHE 7, ARG 6, ILE 44, ARG 42, ASN 8
	Levodopa	PHE 7, LEU 26, VAL 3, ILE 44	-4.49	4	ARG 42, ARG 6, PHE 4, ASN 8	PHE 7, LEU 26, VAL 3, ILE 44, VAL 5, VAL 43, ARG 42, ARG 6, PHE 4, ASN 8
6GLC_B (Fig. 3d)	2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone	VAL 26, PHE 4, PHE 45, ILE 3, LEU 56, ILE 61, LEU 50	-7.52	2	ILE 44, LYS 6	VAL 26, PHE 4, PHE 45, ILE 3, LEU 56, ILE 61, LEU 50, VAL 5, LEU 43, LYS 6, ILE 44
	3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl) methylene]-, (E)-	LEU 50, ARG 42, THR 7, LYS 6, PHE 4, GLU 64, PHE 45	-7.28	1	ILE 44	LEU 50, ARG 42, THR 7, LYS 6, PHE 4, GLU 64, PHE 45, VAL 5, ILE 61, LEU 43, ILE 3, LEU 8, ILE 44
	4, 4′-Dimethoxychalcone	ILE 3, LYS 6, GLU 64, ASN 60, GLN 62, LEU 50	-7.3	2	PHE 4, ILE 44	ILE 3, LYS 6, GLU 64, ASN 60, GLN 62, LEU 50, ILE 61, PHE 45, VAL 5, LEU 43, PHE 4, ILE 44
	Ketone,7-methoxy-2-benzofuranyl methyl	LEU 50, ILE 44, VAL 5, GLU 64, PHE 45	-5.68	1	PHE 4	LEU 50, ILE 44, VAL 5, GLU 64, PHE 45, LEU 43, ILE 3, ILE 61, LYS 6, PHE 4
	Levodopa	GLU 16	-5.18	3	ASP 32, THR 14, LYS 29	GLU 16, LEU 15, LYS 33, ASP 32, THR 14, LYS 29
5VBO (Fig. 4a)	2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone	LYS 16, MET 331, TRP 63, ARG 345, GLU 154	-6.29	2	ASP 66, ARG 67	LYS 16, MET 331, TRP 63, ARG 345, GLU 154, ALA 64, TYR 156, GLU 112, PRO 155, TRP 341, ASP 66, ARG 67
	3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl) methylene]-, (E)-	LEU 92, PRO 82, MET 107, ASP 106, MET 105, TYR 97, ASN 135	-5.71	1	ASN 140	LEU 92, PRO 82, MET 107, ASP 106, MET 105, TYR 97, ASN 135, TYR 139, LEU 94, ILE 146, MET 132, PHE 83, VAL 87, CYS 136, ASN 140
	4, 4′-Dimethoxychalcone	PRO 48, ASN 44, ASP 88, PRO 45, TYR 98, PRO 47, TYR 97, MET 105, ASP 106, ASN 135	-7.84	1	PRO 46	PRO 48, ASN 44, ASP 88, PRO 45, TYR 98, PRO 47, TYR 97, MET 105, ASP 106, ASN 135, PRO 104, PRO 86, VAL 87, PRO 46
	Ketone,7-methoxy-2-benzofuranyl methyl	ILE 101, ASN 1356, MET 132, PHE 83	-5.71	2	TYR 97, ASN 140	ILE 101, ASN 1356, MET 132, PHE 83, CYS 136, VAL 87, LEU 92, ILE 146, TYR 139, LEU 94, TYR 97, ASN 140
	Levodopa	LEU 94, TYR 139, ILE 100, PRO 95, LYS 141	-4.39	2	ASP 96, ASP 144	LEU 94, TYR 139, ILE 100, PRO 95, LYS 141, ASN 140, ASP 96, ASP 144

(*Contd.*)

Table 7 — Binding energy of various flavonoid compounds for *Pongamia pinnata* (*Contd.*)

Gene	Ligand	Vanderwal's interaction	Binding energy	No. of hydrogen bonds	Hydrogen interactions	Total no of residues
	2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone	GLU 94, LYS 63, TYR 67, LEU 7, GLN 95, ARG 98	-6.97	2	LYS 62, GLY 65	GLU 94, LYS 63, TYR 67, LEU 7, GLN 95, ARG 98, PRO 66, ILE 91, VAL 70, LYS 62, GLY 65
	3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl) methylene]-, (E)-	GLY 65, ARG 98, GLN 95, VAL 70	-6.13	Nil	Nil	GLY 65, ARG 98, GLN 95, VAL 70, GLU 94, ASN 97, PRO 66, ILE 91, LYS 62, LEU 7, TYR 67
3EZG (Fig. 4b)	4,4′-Dimethoxychalcone	GLY 65, GLN 95, VAL 70	-6.46	2	ASN 97, ARG 98	GLY 65, GLN 95, VAL 70, GLU 94, PRO 66, TYR 67, ILE 91, LEU 7, LYS 62, ASN 97, ARG 98
	Ketone,7-methoxy-2-benzofuranyl methyl	ARG 98, GLN 95, VAL 70, GLY 65, LYS 63	-6.21	2	LYS 62	ARG 98, GLN 95, VAL 70, GLY 65, LYS 63, ILE 91, PRO 66, LEU 7, TYR 67, GLU 94, LYS 62
	Levodopa	LEU 38, ALA 11, GLY 37, PRO 54, ASP 42	-5.11	5	LEU 10, GLY 40, LYS 12, PRO 43, GLN 45	LEU 38, ALA 11, GLY 37, PRO 54, ASP 42, ALA 39, VAL 44, LEU 10, GLY 40, LYS 12, PRO 43, GLN 45
	2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone	GLU 207, PHE 208, ARG 191, ILE 188, ASN 190, ASP 185, PRO 189, VAL 186, ALA 225, PHE 210	-6.08	1	LEU 187	GLU 207, PHE 208, ARG 191, ILE 188, ASN 190, ASP 185, PRO 189, VAL 186, ALA 225, PHE 210, PHE 209, LEU 187
	3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl) methylene]-, (E)-	VAL 186, PHE 208, ALA 206, SER 167, PHE 209, PHE 210, PRO 223, VAL 224	-5.71	2	ALA 225, ASN 190	VAL 186, PHE 208, ALA 206, SER 167, PHE 209, PHE 210, PRO 223, VAL 224, LEU 187, GLU 207, PRO 189, ALA 225, ASN 190
4I1F (Fig. 4c)	4,4′-Dimethoxychalcone	LYS 413, GLU 409, SER 407, LYS 408, THR 234, SER 233, ASN 448	-5.57	1	LYS 416	LYS 413, GLU 409, SER 407, LYS 408, THR 234, SER 233, ASN 448, TRP 447, MET 432, LYS 412, THR 414, LYS 416
	Ketone,7-methoxy-2-benzofuranyl methyl	SER 167, GLU 207, ALA 206, ASP 185, ILE 188, VAL 186, PHE 208, LEU 187	-5.84	1	ASN 190	SER 167, GLU 207, ALA 206, ASP 185, ILE 188, VAL 186, PHE 208, LEU 187, PRO 189, MET 192, ARG 191, ASN 190
	Levodopa	VAL 186, LEU 187, PHE 210, PHE 209, GLU 207, SER 167, ASN 190, SER 205, ARG 191, MET 192, ILE 188, PRO 189	-4.77	2	PHE 208, ALA 206	VAL 186, LEU 187, PHE 210, PHE 209, GLU 207, SER 167, ASN 190, SER 205, ARG 191, MET 192, ILE 188, PRO 189, PHE 208, ALA 206

(*Contd.*)

Table 7 — Binding energy of various flavonoid compounds for *Pongamia pinnata* (*Contd.*)

Gene	Ligand	Vanderwal's interaction	Binding energy	No. of hydrogen bonds	Hydrogen interactions	Total no of residues
3BQP_A (Fig. 4d)	2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone	CYS 78, CYS 5, TYR 54, PRO 79, GLY 76	-6.37	5	ALA 77, PHE 4, GLY 2, ASP 1, GLY 3	ALA 77, PHE 4, GLY 2, ASP 1, GLY 3, CYS 78, CYS 5, TYR 54, PRO 79, GLY 76
	3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl)methylene]-, (E)-	GLU 6, PRO 68, LEU 39, ASN 18	-5.84	1	ARG 17	GLU 6, PRO 68, LEU 39, ASN 18, LYS 10, LYS 9, GLY 13, PHE 38, TYR 14, ARG 17
	4,4′-Dimethoxychalcone	SER 69, ARG 17, PHE 38, PRO 40	-5.57	1	LYS 10	SER 69, ARG 17, PHE 38, PRO 40, LYS 9, PRO 68, GLY 13, LEU 39, TYR 14, LYS 10
	Ketone,7-methoxy-2-benzofuranyl methyl	CYS 47, TYR 43, GLN 44, PRO 42, ASP 41	-4.98	2	GLN 46, LYS 45	CYS 47, TYR 43, GLN 44, PRO 42, ASP 41, GLN 46, LYS 45
	Levodopa	GLY 3, CYS 5, TYR 54, GLY 2, PRO 79, CYS 78	-6.01	4	ASP 1, PHE 4, ALA 77, GLY 76	GLY 3, CYS 5, TYR 54, GLY 2, PRO 79, CYS 78, ASP 1, PHE 4, ALA 77, GLY 76
3BQP_B (Fig. 5a)	2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone	TYR 43, GLN 46, PHE 4, GLY 2	-7.12	4	GLY 3, VAL 7, GLU 6, CYS 5	GLY 3, VAL 7, GLU 6, CYS 5, TYR 43, GLN 46, PHE 4, GLY 2
	3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl) methylene]-, (E)-	PHE 38, GLY 13, SER 69	-5.76	1	ARG 17	TYR 14, LEU 39, LYS 10, LYS 9, PRO 68, PHE 38, GLY 13, SER 69, ARG 17
	4,4′-Dimethoxychalcone	TYR 14, LEU 39, PRO 40, PHE 38, PRO 68, GLU 6, GLY 13	-5.6	2	ARG 17, LYS 9	TYR 14, LEU 39, PRO 40, PHE 38, PRO 68, GLU 6, GLY 13, LYS 10, ARG 17, LYS 9
	Ketone,7-methoxy-2-benzofuranyl methyl	GLY 13, PRO 68, LYS 9, PHE 38	-5.31	1	ARG 17	GLY 13, PRO 68, LYS 9, PHE 38, PRO 40, LYS 10, LEU 39, TYR 14, ARG 17
	Levodopa	GLN 49, TYR 54, GLY 3, GLY 2, CYS 5	-5.72	4	ALA 77, PHE 4, GLY 76, GLU 53	GLN 49, TYR 54, GLY 3, GLY 2, CYS 5, ASP 1, ALA 77, PHE 4, GLY 76, GLU 53
3Q27 (Fig. 5b)	2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone	LYS 16, MET 331, TRP 63, GLU 154, ARG 345	-6.85	2	ARG 67, ASP 66	LYS 16, MET 331, TRP 63, GLU 154, ARG 345, TYR 156, ALA 64, GLU 112, PRO 155, TRP 341, ASP 66, ARG 67
	3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl)methylene]-, (E)-	TYR 177, ILE 179, ASP 96, TYR 172, GLY 175, MET 331, HIS 65, PRO 332, ALA 97	-6.6	2	LYS 176, ASN 333	TYR 177, ILE 179, ASP 96, TYR 172, GLY 175, MET 331, HIS 65, PRO 332, ALA 97, ILE 334, PHE 93, ILE 330, LYS 176, ASN 333
	4,4′-Dimethoxychalcone	PHE 170, TYR 168, TYR 177, ALA 97, ASP 96, PRO 332	-5.95	1	ALA 169	PHE 170, TYR 168, TYR 177, ALA 97, ASP 96, PRO 332, TYR 172, ILE 330, PHE 259, TRP 159, GLU 329, ALA 169

(*Contd.*)

Table 7 — Binding energy of various flavonoid compounds for *Pongamia pinnata* (*Contd.*)

Gene	Ligand	Vanderwal's interaction	Binding energy	No. of hydrogen bonds	Hydrogen interactions	Total no of residues
	Ketone,7-methoxy-2-benzofuranyl methyl	ILE 330, TYR 177, PHE 170, TYR 172, GLU 329, TYR 168	-5.88	2	ALA 169, LYS 171	ILE 330, TYR 177, PHE 170, TYR 172, GLU 329, TYR 168, TRP 159, PHE 259, PRO 332, ALA 169, LYS 171
	Levodopa	ARG 345, TRP 341, GLU 45, MET 331, GLY 261, TRP 231, LYS 16	-5.14	4	GLU 154, ARG 67, ASP 66, GLU 112	ARG 345, TRP 341, GLU 45, MET 331, GLY 261, TRP 231, LYS 16, TRP 63, ALA 64, TYR 156, GLU 154, ARG 67, ASP 66, GLU 112
CHCHCD2 (Fig. 5c)	2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone	ALA 79, HIS 78, GLY 82	-5.54	3	GLY 77, THR 81, ILE 80	ALA 79, HIS 78, GLY 82, GLY 77, THR 81, ILE 80
	3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl) methylene]-, (E)-	GLN 100, ASP 96, THR 98, GLU 101	-3.81	Nil	Nil	GLN 100, ASP 96, THR 98, GLU 101, TYR 99, PRO 102, ILE 97
	4,4′-Dimethoxychalcone	GLN 111, ALA 108, GLN 110, GLN 109, LEU 115	-3.31	1	GLN 112	GLN 111, ALA 108, GLN 110, GLN 109, LEU 115, GLN 112, ILE 118, LEU 122
	Ketone,7-methoxy-2-benzofuranyl methyl	ALA 63, ALA 60, GLN 58	-3.76	2	THR 62, THR 61	ALA 63, ALA 60, GLN 58, MET 59, THR 62, THR 61
	Levodopa	Nil	-2.0	3	LYS 119, GLU 123, GLN 126	LYS 119, GLU 123, GLN 126
	2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone	GLY 225, ASP 192, ARG 223, PHE 160, ILE 159, ARG 381, ALA 247	-6.33	4	VAL 161, GLU 163, HIS 248, TYR 386	GLY 225, ASP 192, ARG 223, PHE 160, ILE 159, ARG 381, ALA 247, LEU 226, ASP 246, VAL 161, GLU 163, HIS 248, TYR 386
	3(2H)-Benzofuranone, 6-methoxy-2-((3-methoxyphenyl) methylene)-(E)-	ARG 381, ASP 246, TYR 386, ILE 159, ASP 192, GLY 225, VAL 349, TYR 350	-6.05	1	ARG 223	ARG381, ASP 246, TYR 386, ILE 159, ASP 192, GLY 225, VAL 349, TYR 350, VAL 161, LEU 226, HIS 248, LYS 384, HIS 378, ARG 223
WBSCR17 (Fig. 5d)	4,4′-Dimethoxychalcone	ALA 489, THR 488, LEU 581, ASP 580, LEU 491, GLY 578, TYR 492	-4.73	Nil	Nil	ALA 489, THR 488, LEU 581, ASP 580, LEU 491, GLY 578, TYR 492, ILE 579, PRO 493, VAL 477, ILE 490, LEU 535, PRO 536
	Ketone,7-methoxy-2-benzofuranyl methyl	PHE 160, HIS 248, ASP 246, TYR 386, TYR 350, LYS 384, VAL 349, ARG 223, ASP 192	-5.31	1	LEU 226	PHE 160, HIS 248, ASP 246, TYR 386, TYR 350, LYS 384, VAL 349, ARG 223, ASP 192, ILE 159, VAL 161, LEU 226
	Levodopa	GLY 254, ALA 253, LEU 145, LYS 149	-3.44	4	ARG 138, LYS 143, GLU 257, TYR 147	GLY 254, ALA 253, LEU 145, LYS 149, LYS 146, ARG 138, LYS 143, GLU 257, TYR 147

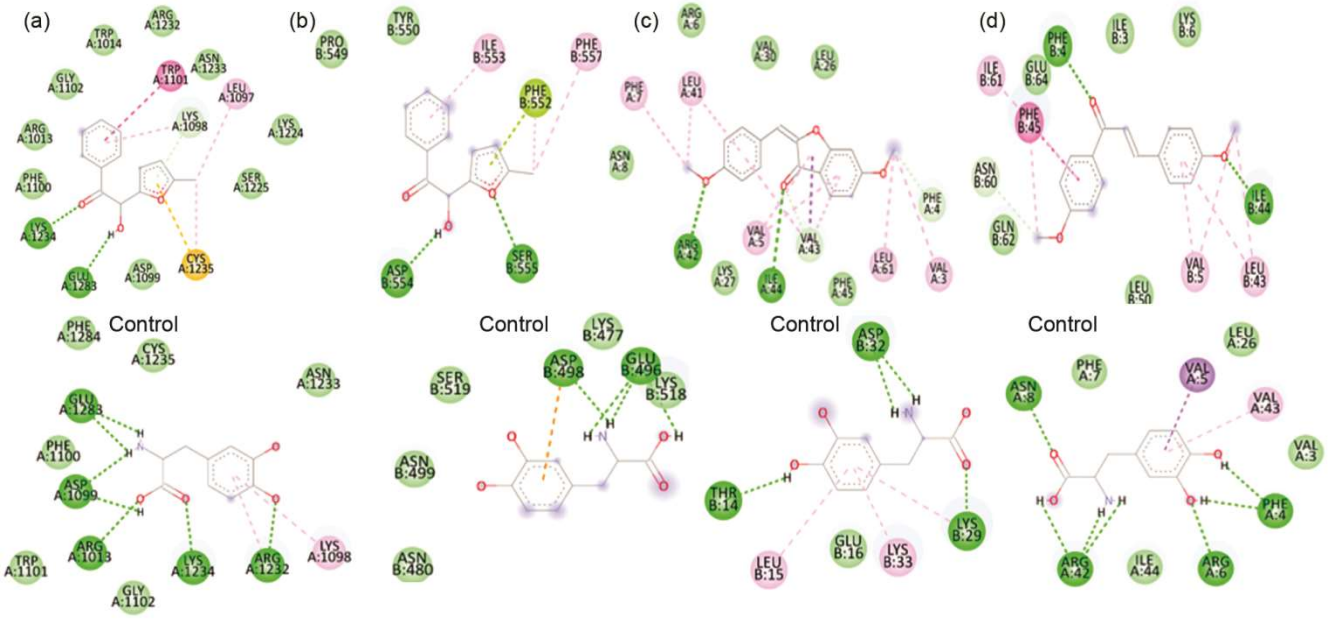


Fig. 3 — (a) 2D interaction of ligand 3(2H)-Benzofuranone, 6-methoxy-2((4-methoxyphenyl)methylene)-, (E)- and control levodopa with 6GLC_A; (b) 2D interaction of ligand 2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone and control levodopa with 6ES1_B; (c) 2D interaction of ligand 3(2H)-Benzofuranone, 6-methoxy-2((3-methoxyphenyl)methylene)-, (E)- and control levodopa with 6GLC_A; and (d) 2D interaction of ligand 4,4′-Dimethoxychalnone and control levodopa with 6GLC_B

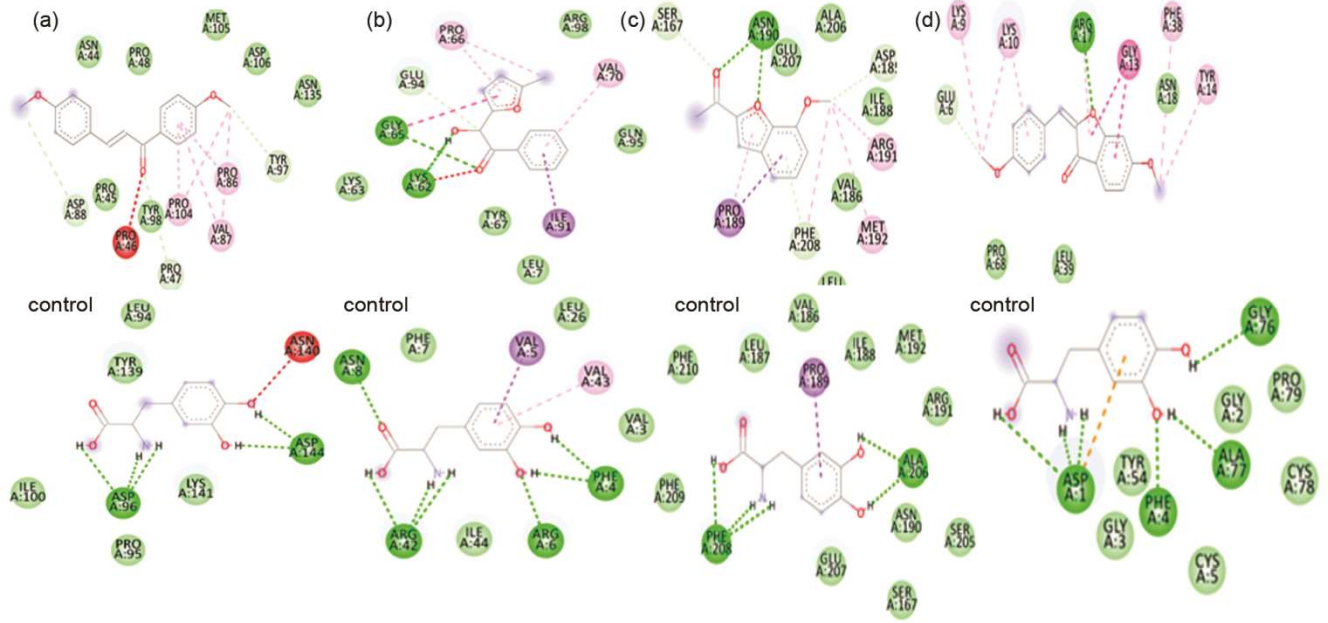


Fig. 4— (a) 2D interaction of ligand 4,4′-Dimethoxychalnone and control levodopa with 5VBO; (b) 2D interaction of ligand 2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone,control levodopa with 3EZG; (c) 2D interaction of ligand Ketone, 7-methoxy-2-benzofuranyl methyl and control levodopa with 411F; and (d) 2D interaction of ligand 2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone and control levodopa with 3BQP_A

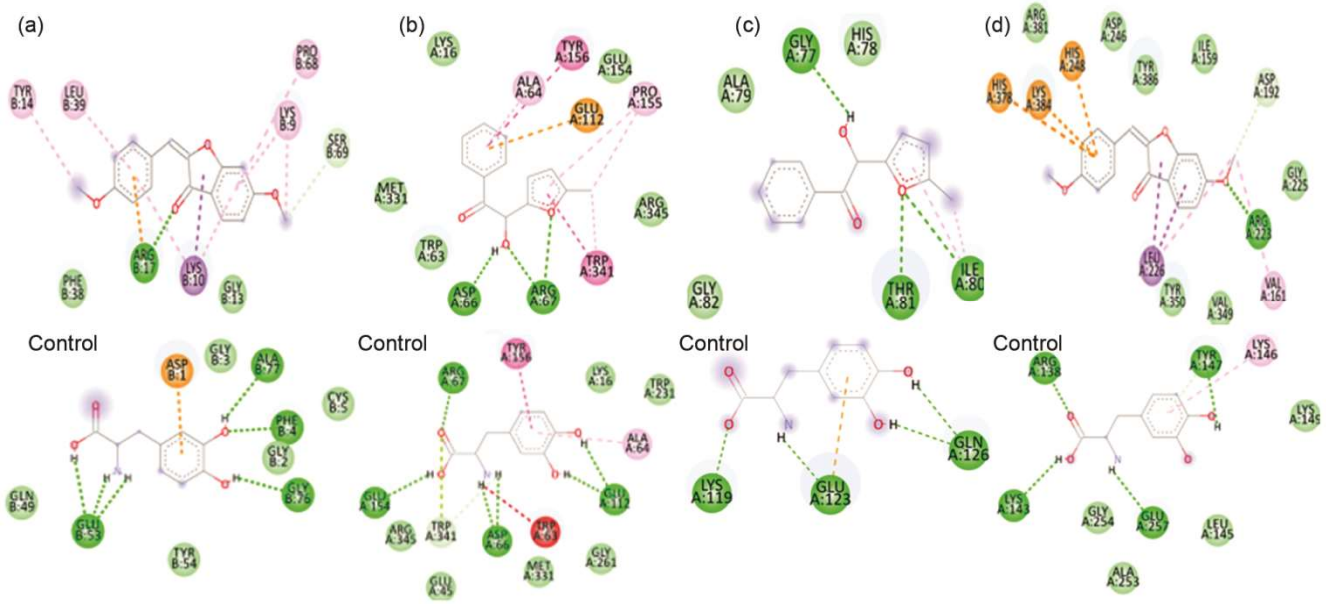


Fig. 5— (a) 2D interaction of ligand 3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl)methylene]-, (E)-3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl)methylene]-, (E)-3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl)methylene]-, (E)-and control levodopa with 3BQP_B; (b) 2D interaction of ligand 2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone and control levodopa with 3Q27; (c) 2D interaction of ligand 2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone and control levodopa with CHCHCD2; and (d) 2D interaction of ligand 3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl)methylene]-, (E)-3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl)methylene]-, (E)-3(2H)-Benzofuranone, 6-methoxy-2-[(3-methoxyphenyl)methylene]-, (E)-and control levodopa with WBSCR17

of PINK1 gene mutations producing selective death of nerve cells defining Parkinson's disease is not known[35]. The loss of these cells reduces connection between the brain and muscles, so the brain loses control over muscle movement subsequently. This mitochondrial dysfunction contributes to oxidative stress, energy depletion, and ultimately the degeneration of dopamine-secreting neurons in Parkinson's disease[36,37]. Comparatively not only with PINK1 gene, 2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone showed good activity among all gene like LRRK2, PARK7 and SNCA (Figs. 4 and 5).

DPPH ASSAY

The antioxidant and free radical scavenging properties of the aqueous extract derived from the plant were evaluated against 1,1-diphenyl-2-picrylhydrazyl (DPPH). Quercetin and L-ascorbic acid served as positive controls, with the final absorbance readings taken at 517 nm[38]. Both the plant extracts demonstrated enhanced inhibition percentages at the 500 µg/mL concentration, with *Pongamia pinnata* displaying 54.28% inhibition. Thus, the selected plant extract possessed significant potential as a natural antioxidant source, thereby supporting its traditional use in medicine (Fig. 6a).

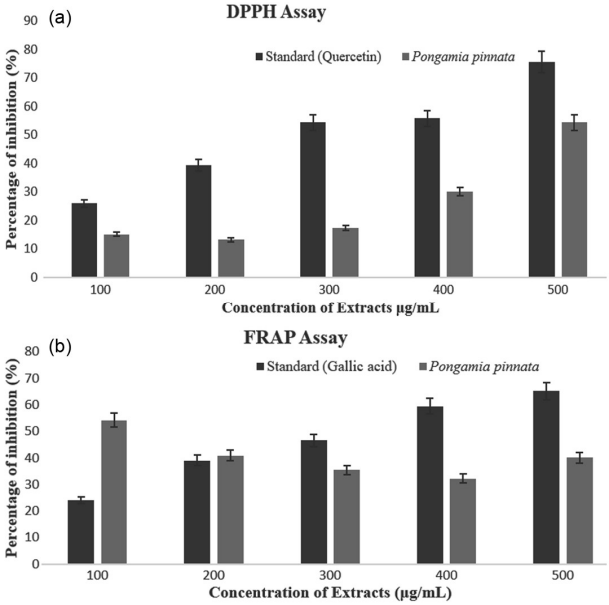


Fig. 6 — (a) DPPH; and (b) FRAP assay activity of *Pongamia pinnata* ethanol extract

FRAP assay

The antioxidant activity of the plant extracts was evaluated using the FRAP assay. This method uses electron-donating antioxidants to convert the colourless Fe^{3+}-TPTZ complex to the blue Fe^{2+}-

tripyridyl triazine complex in acidic conditions. The powdered samples were evaluated with gallic acid as a standard[39]. Measurements of absorbance were conducted at 510nm, and the average values were used to calculate the antioxidant inhibitory capacity of the compounds[40]. As shown in the Figure 6b, at a concentration of 100 µg/mL, *Pongamia pinnata* showed significant inhibition. As a result, this analysis suggests that both plant extracts have significantly higher antioxidant potential than the standard (gallic acid).

Conclusion

The neuroprotective effects of *Pongamia pinnata* extracts have presented promising results in preliminary studies. Experimental reports of neurological related disorders, including Parkinson's and Alzheimer disease have demonstrated neuroprotection and anti-inflammatory properties in certain compounds extracted from *Pongamia pinnata*. Nevertheless, these results are provisional, and further research is necessary to ensure the safety and efficacy of *Pongamia pinnata* in humans with neurological disorders. It is imperative to exercise caution when employing *Pongamia pinnata* or any other herbal remedy for neurological disorders. The potential of flavonoid compounds in *Pongamia pinnata* to treat these disorders was underscored by our research. *Pongamia pinnata*, a novel plant in the context of neurodegenerative disorders, exhibited promising antioxidant and binding capabilities, as demonstrated by DPPH and FRAP assays. These results indicate that they have the potential to impede the activity of mutated genes that are involved in the development of neurodegenerative disorders. The top binding compounds from *Pongamia pinnata* were 2-Hydroxy-2-(5-methylfuran-2-yl) 1-Phenylethanone, which exhibited significant binding across multiple genes in the neurodegenerative pathway. These results indicate that *Pongamia pinnata* may be a promising source of novel treatments for neurodegenerative diseases. Consequently, this compound may be examined for potential medicinal applications, such as in the development or discovery of drugs.

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Conflict of interest

All authors declare no conflicts of interest.

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