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In Silico Approach of Neuroprotective Mechanisms of Piper Beetle Leaves Derived Nutraceutical Phytochemicals in Parkinson's Disease

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Abstract: ***Parkinson's disease (PD) is a rapidly increasing neurodegenerative disorder and a major global health concern. While aging is the primary risk factor, modern dietary patterns, including high consumption of processed foods and associated metabolic disturbances, may contribute to oxidative stress, chronic inflammation, and neuronal dysfunction implicated in PD pathogenesis. In this context, plant-derived bioactive compounds have emerged as promising neuroprotective agents. This study explored the therapeutic potential of* Piper betle*, a traditional functional food and medicinal plant, against PD using an integrated network pharmacology and molecular docking approach. A total of 100 phytochemicals from* P. betle *were screened for drug-likeness and pharmacokinetic properties. ADME analysis showed that 89% of compounds satisfied Lipinski's Rule of Five, indicating favorable oral bioavailability and drug-like properties. Key compounds, including eugenol, hydroxychavicol, caffeic acid, ferulic acid, squalene, and vitamin E, exhibited strong blood–brain barrier permeability and central nervous system accessibility with minimal cytochrome P450 inhibition. Target prediction and disease association analysis identified 19 common targets associated with key PD mechanisms, including dopaminergic neurotransmission, apoptosis, neuroinflammation, and survival signaling pathways. Protein–protein interaction analysis highlighted TP53, AKT1, and MAPK1 as major hub proteins. Functional enrichment analysis showed significant associations with Parkinson's disease pathways, dopaminergic synapse, apoptosis, and neuroprotective signaling cascades. Molecular docking studies targeting tyrosine hydroxylase revealed strong binding affinities, with flavoquinone exhibiting the highest binding energy (−7.8 kcal/mol).***

Keywords: Piper beetle, Neutaceutical, Phytochemical, In silico, Parkinson's Disease and Lewy bodies

1. Introduction

Parkinson's disease (PD) is one of the most prevalent neurodegenerative disorders worldwide, characterized by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to dopamine deficiency and Lewy body accumulation (Karakaya and Demir, 2025). Clinically, PD presents with motor symptoms such as bradykinesia, resting tremor, rigidity, and postural instability, along with non-motor features including cognitive impairment, sleep disturbances, and neuropsychiatric dysfunction (Pirtošek and Trošt, 2023). Its pathogenesis involves multifactorial mechanisms including oxidative stress, mitochondrial dysfunction, neuroinflammation, protein aggregation, and impaired cellular homeostasis (Bhong et al., 2024).

The global burden of PD is rising rapidly, affecting over 6 million individuals and projected to exceed 12 million by 2040 due to population aging (Sun et al., 2025; Su et al., 2025). Current therapies, including levodopa and dopamine agonists, provide symptomatic relief but do not halt disease progression and are associated with long-term complications and adverse effects (Hansen et al., 2022; Devi et al., 2024). These limitations highlight the need for safer, multi-target therapeutic strategies.

Tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine biosynthesis, is a key therapeutic target in PD due to its central role in dopamine homeostasis (Du et al., 2022; Kaczor et al., 2022).

Medicinal plants offer promising neuroprotective compounds capable of modulating multiple disease pathways (Cheshomi et al., 2021). *Piper betle* L. is traditionally used in Asian medicine and contains over 100 bioactive compounds, including phenylpropanoids, flavonoids, terpenoids, and phenolic acids with antioxidant and neuroprotective activities (Biswas et al., 2022; Bae et al., 2025).

This study applies integrated network pharmacology and molecular docking to explore the neuroprotective potential of *P. betle* against PD, focusing on multi-target mechanisms and tyrosine hydroxylase, providing a systems-level understanding of its therapeutic potential.

2. Materials and Methods

Research Design: An integrated computational framework combining network pharmacology, bioinformatics, and molecular docking was employed to evaluate the neuroprotective potential of *Piper betle* phytochemicals against Parkinson's disease (PD). The workflow included phytochemical screening, ADME and drug-likeness evaluation, target prediction, disease–gene association analysis, PPI network construction, functional enrichment analysis, and molecular docking validation (Lata et al., 2025).

Phytochemical Collection and Drug-Likeness Analysis: Phytochemicals of *Piper betle* were retrieved from Dr. Duke's Phytochemical and Ethnobotanical Database and literature sources (Pradhan and Kulkarni, 2024; Biswas et al., 2022). Structural data, PubChem IDs, and SMILES were obtained from PubChem and cross-verified using ChemSpider and HMDB (Cheng et al., 2014; Szilágyi et al.,

2021). Drug-likeness and physicochemical properties were assessed using SwissADME based on Lipinski's Rule of Five (Ntie-Kang et al., 2019; Nhlapho et al., 2024; Bhatkhande et al., 2025).

ADME and Target Prediction: ADME properties including intestinal absorption, BBB permeability, CYP interactions, and clearance were predicted using pkCSM (Yeni and Rachmania, 2022; Chen et al., 2012; Raunio et al., 2015). Target prediction was performed using SwissTargetPrediction and STITCH, and gene symbols were standardized via UniProt and HGNC (Mayr et al., 2020; Antonisamy et al., 2024; Kamepalli et al., 2025).

Parkinson's Disease Target Identification and Network Analysis: PD-related genes were retrieved from GeneCards and OMIM, and overlapping targets with *P. betle* compounds were identified using Venn analysis (Mayr et al., 2020; Chowdhury and Vijaykumar, 2025). The intersecting targets were used to construct a PPI network via STRING and visualized in Cytoscape 3.9.1. Network topology and hub genes were identified using NetworkAnalyzer and CytoHubba algorithms including Degree, Betweenness, Closeness, MCC, DMNC, and EPC (Borgatti and Everett, 2006; Chuang and Kung, 2005; Elmezain et al., 2021).

Functional Enrichment Analysis: GO and KEGG pathway enrichment analyses were performed using the DAVID database to identify significantly enriched biological processes, molecular functions, cellular components, and pathways. Terms with FDR < 0.05 were considered statistically significant (Chowdhury and Vijaykumar, 2025).

Molecular Docking: Molecular docking was conducted to validate interactions between key phytochemicals and tyrosine hydroxylase (TH; PDB ID: 1TOH), the rate-limiting enzyme in dopamine biosynthesis. Docking was performed using AutoDock Vina 1.1.2 with a rigid receptor and flexible ligands. Binding affinities and interaction profiles were analyzed to assess the therapeutic potential of *P. betle* compounds against PD (Ferreira et al., 2015; Lata et al., 2025).

Table 1: List of ligand molecules selected from the *Piper betle*

S. No	Compound Name	S. No	Compound Name
1	2-Methoxy-4–2-Propenyl	51	Elemene
2	Eugenol	52	Icariside D1
3	Flavoquinone	53	Indanol
4	4-Allyl-1,2-Diacetoxybenzene	54	Pachypodol
5	Hydroxychavicol	55	Thujene
6	Methyl Eugenol	56	Germacrene D
7	Chavibetol	57	Camphene
8	4-Chromanol	58	Myrcene
9	Chavicol	59	Thymol
10	Safrole	60	Estragole,
11	Estragole	61	Chavibetol Methyl Ether
12	Anethole	62	Apigenin
13	Isoeugenol	63	Quercetin
14	Allylpyrocatechol	64	Phenylpropanoid
15	Piperbetol	65	Phenol, 2-Methoxy-3-(2-Propenyl)-
16	Arecoline	66	Lignan
17	Carvacrol	67	Oleanolic Acid
18	Caryophyllene	68	4-Nitroisopropylbenzene
19	Piperitol	69	Benzoic Acid, 2,4-Dimethyl-
20	Linalool	70	Delta-Cadinene
21	Sabinene	71	Nerolidol
22	Cepharadione	72	P-Cymene
23	Piperlonguminine	73	Myristic Acid
24	1,8-Cineole	74	Alpha-Cadinol
25	Ursolic Acid	75	Androstan-17-One, 3-Ethyl-3-Hydroxy-, (5 Alpha
26	Caffeic Acid	76	Beta.-Sitosterol
27	Ferulic Acid	77	Glycerol
28	Limonene	78	Hexadecanoic Acid, Methyl Ester
29	Piperine	79	Stigmasta-3,5-Diene
30	Squalene	80	Humulane-1, 6-Dien-3-Ol
31	3-Allyl-6-Methoxyphenol	81	(2e,4e)-N-Isobutylhexadeca-2,4-Dienamide
32	4-Allyl-2-Methoxy-Phenolacetate	82	Bicyclo [7.2.0] Undec-4-Ene Derivative
33	Cyclohexene	83	9,12-Octadecadienoic Acid
34	2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4h-Pyran-4-One	84	Silane, Chlorodimethyl
35	Phytol	85	Ethyl Alcohol
36	Guaiac Acetate	86	Methionine
37	Isophytol	87	3,5-Dimethylbenzoic Acid
38	2-Pentylfuran	88	Phthalic Acid
39	4-Vinyl Guaiacol	89	Cyclopropyl [3,4-Dimethoxyphenyl] Methanone
40	Hydrazine, 1,2-Dimethyl-	90	Octadecadienal
41	Terpene	91	Tocopherol
42	Cineol	92	Torreyol

43	Menthone	93	Benzenamine
44	Isosafrol	94	Benzenamine, 4,4' - (1,2- Ethenediyl) Bis-
45	Gentisic Acid	95	1- Methylphenazine 5-Oxide
46	Vitexin 2″-*O*-Rhamnoside.	96	Vitamin E
47	Ethyl Aminomethylformimidate	97	Cyclohexen-1-One
48	Propanoic Acid, 2-Hydroxy-	98	Phytol Acetate
49	1,2-Cyclopentanedione	99	Isopropyl Myristate
50	Erigeside Ii	100	Viridiflorol

2.1 Chemical Classification of Identified Compounds

The 100 identified compounds represent diverse phytochemical classes of *P. betle*. **Phenylpropanoids** form the major group, including eugenol, chavibetol, hydroxychavicol, chavicol, methyl eugenol, isoeugenol, safrole, estragole, anethole, and allylpyrocatechol. These C6–C3 derivatives arise from the shikimate/phenylpropanoid pathway and exhibit strong antioxidant, anti-inflammatory, and neuroprotective activities, with eugenol being the most studied bioactive constituent (Vogt, 2010; Kabuto et al., 2007; Nagababu et al., 2010; Pramod et al., 2010).

Terpenes and terpenoids include monoterpenes, sesquiterpenes, and triterpenes such as β-caryophyllene, germacrene D, squalene, ursolic acid, and oleanolic acid. These compounds are synthesized via the mevalonate or MEP pathways and exhibit diverse biological activities. β-caryophyllene acts as a CB2 receptor agonist with anti-inflammatory and neuroprotective effects, while ursolic and oleanolic acids demonstrate mitochondrial and neuroprotective properties (Gershenzon & Dudareva, 2007; Gertsch et al., 2008; Jiao et al., 2025).

Flavonoids include apigenin, quercetin, and vitexin 2″-O-rhamnoside, characterized by a C6–C3–C6 structure and strong antioxidant and signaling-modulatory properties. Quercetin and apigenin have shown significant neuroprotective effects in Parkinson's disease models (Panche et al., 2016; Ay et al., 2017; Patil et al., 2003).

Phenolic acids such as caffeic acid, ferulic acid, and gentisic acid exhibit potent antioxidant and neuroprotective effects, protecting against oxidative stress–mediated neuronal damage. CAPE has also demonstrated anti-inflammatory and anti-apoptotic effects in PD models (Thapak et al., 2013; Fontanilla et al., 2011).

Alkaloids and nitrogenous compounds include arecoline and piperine, with piperine known for enhancing bioavailability and exhibiting neuroprotective properties (Chonpathompikunlert et al., 2010).

Fatty acids and sterols such as myristic acid, hexadecanoic acid methyl ester, β-sitosterol, and phytol contribute to anti-inflammatory and neuroprotective activity, with β-sitosterol showing cholesterol-mimicking and neuroprotective effects (Loizou et al., 2010).

2.2 Physicochemical Property Assessment

Physicochemical properties of all 100 compounds were evaluated to assess drug-likeness and oral bioavailability (Table 2). Key descriptors included molecular weight (MW), lipophilicity (LogP), hydrogen bond acceptors (HBA), and hydrogen bond donors (HBD), forming the basis of Lipinski's Rule of Five for predicting oral drug suitability (Lipinski et al., 2001).

Table 2: Predicted Physicochemical property of *Piper betle* compounds

Compound Name	Molecular Formula	Molecular Weight (g/mol)	HBA	HBD	LogP
Flavoquinone	C10H6N4O2	214.18	4	2	0.14
4-Allyl-1,2-Diacetoxybenzene	C13H14O4	234.25	4	0	-0.21
Hydroxychavicol	$C_9H_{10}O_2$	150.17	2	2	-0.07
Methyl Eugenol	C11H14O2	178.23	2	0	-0.09
Chavibetol	C10H12O2	164.2	2	1	-0.08
4-Chromanol	C9H10O2	150.17	2	1	0.17
Chavicol	C9H10O	134.17	1	1	0.78
Safrole	$C_{10}H_{10}O_2$	162.18	2	1	1.35
Estragole	C10H12O	148.2	1	0	0.67
Anethole	C10H12O	148.2	1	0	0.35
Isoeugenol	C10H12O2	164.2	2	1	-0.39
Allylpyrocatechol	C9H10O2	150.17	2	2	0.71
Piperbetol	$C_{22}H_{26}O_6$	386.4	6	1	-2.4
Arecoline	$C_8H_{13}NO_2$	155.19	3	0	-2.78
Carvacrol	$C_{10}H_{14}O$	150.22	1	1	-0.43
Caryophyllene	C15H24	204.35	0	0	-1.02
Piperitol	C20H20O6	356.4	6	1	-0.47
Linalool	C10H18O	154.25	1	1	-2.04
Sabinene	C10H16	136.23	0	0	-0.39
Cepharadione	C19H15NO4	321.3	4	0	0.74
Piperlonguminine	$C_{16}H_{19}NO_3$	273.33			
1,8-Cineole	C10H18O	154.25	1	0	-1.69
Ursolic Acid	C30H48O3	456.7	3	2	-4.94

Caffeic Acid	C9H8O4	180.16	4	3	-0.91
Ferulic Acid	C10H10O4	194.18	4	2	-0.93
Limonene	C10H16	136.23	0	0	-0.74
Piperine	C17H19NO3	285.34	3	0	-0.1
Squalene	$C_{30}H_{50}$	410.7	0	0	-5.04
3-Allyl-6-Methoxyphenol	C10H12O2	164.2	2	1	-0.08
4-Allyl-2-Methoxy-Phenolacetate	C12H14O4	222.24	4	1	-0.88
Cyclohexene	C_6H_{10}	82.14	0	0	-0.56
2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4h-Pyran-4-One	C6H8O4	144.12	4	2	-2.63
Phytol	C20H40O	296.5	1	1	-2.5
Guaiac Acetate	C17H28O2	264.4	2	0	-2.64
Isophytol	C20H40O	296.5	1	1	-1.85
2-Pentylfuran	C9H14O	138.21	1	0	0.73
4-Vinyl Guaiacol	$C_9H_{10}O_2$	150.17	2	1	0.19
Hydrazine, 1,2-Dimethyl-	C2H8N2	60.1	0	2	-3.21
Terpene	$C_{10}H_{20}O_2$	172.26	2	2	-3.32
Cineol	$C_{10}H_{18}O$	154.25	1	0	-1.69
Menthone	$C_{10}H_{18}O$	154.25	1	0	-0.92
Isosafrol	$C_{10}H_{10}O_2$	162.18	2	0	1.06
Gentisic Acid	C7H6O4	154.12	4	3	-0.05
Ethyl Aminomethylformimidate	C4H10N2O	102.14	1	3	-3.78
Propanoic Acid, 2-Hydroxy-	C3H6O3	90.08	3	2	-2.15
1,2-Cyclopentanedione	$C_5H_6O_2$	98.10	2	0	-1.12
Erigeside II	$C_{17}H_{24}O_8$	356.4	8	4	-3.95
Elemene	C15H24	204.35	0	0	-0.86
Icariside D1	C19H28O10	416.4	10	6	-6
Indanol	C9H10O	134.17	1	1	0.29
Pachypodol	C18H16O7	344.3	7	2	1.02
Thujene	$C_{10}H_{16}$	136.23	0	0	-0.8
Germacrene D	C15H24	204.35	0	0	-0.2
Camphene	C10H16	136.23	0	0	-0.15
Myrcene	C10H16	136.23	0	0	-0.67
Thymol	C10H14O	150.22	1	1	-0.13
Estragole,	$C_{10}H_{12}O$	148.20			
Chavibetol Methyl Ether	C11H14O2	178.23	2	0	-0.09
Apigenin	C15H10O5	270.24	5	3	1.59
Quercetin	$C_{15}H_{10}O_7$	302.23	7	5	-0.9
Phenylpropanoid	C10H12O2	164.20	2	1	-0.10
Phenol, 2-Methoxy-3-(2-Propenyl)-	C10H12O2	164.2	2	1	0.29
Lignan	C25H30O8	458.5	8	0	-2.3
Oleanolic Acid	C30H48O3	456.7	3	2	-4.99
4-Nitroisopropylbenzene	$C_9H_{11}NO_2$	165.19	2	0	-0.26
Benzoic Acid, 2,4-Dimethyl-	C9H10O2	150.17	2	1	-0.54
Delta-Cadinene	$C_{15}H_{24}$	204.35	0	0	-1.3
Nerolidol	C15H26O	222.37	1	1	-2.81
P-Cymene	C10H14	134.22	0	0	0.23
Myristic Acid	$C_{14}H_{28}O_2$	228.37	2	1	-0.7
Alpha-Cadinol	$C_{15}H_{26}O$	222.37	1	1	-2.24
Androstan-17-One, 3-Ethyl-3-Hydroxy-, (5 Alpha	C21H34O2	318.5	2	1	-3.5
Beta.-Sitosterol	C29H50O4S	494.8	4	1	-4.28
Glycerol	$C_3H_8O_3$	92.09	3	3	-3.24
Hexadecanoic Acid, Methyl Ester	$C_{17}H_{34}O_2$	270.5	2	0	-0.49
Stigmasta-3,5-Diene	C29H48	396.7	2	2	-3.32
Humulane-1, 6-Dien-3-Ol	$C_{15}H_{26}O$	222.37	1	1	-2.19
(2E,4E)-N-Isobutylhexadeca-2,4-Dienamide	C20H37NO	307.5	1	1	-1.03
Bicyclo [7.2.0] Undec-4-Ene Derivative	C15H24	204.35	0	0	-1.02
9,12-Octadecadienoic Acid	C18H32O2	280.4	2	1	-1.73
Silane, Chlorodimethyl	C5H10ClF3Si	190.66	0	0	0.4
Ethyl Alcohol	C2H6O	46.07	1	1	-1.7
Methionine	$C_5H_{11}NO_2S$	149.21	3	3	-3.59
3,5-Dimethylbenzoic Acid	$C_9H_{10}O_2$	150.17	2	1	-0.35
Phthalic Acid	C8H6O4	166.13	4	3	-0.75
Cyclopropyl [3,4-Dimethoxyphenyl] Methanone	$C_{12}H_{14}O_3$	206.24	3	0	-0.5
Octadecadienal	$C_{18}H_{32}O$	264.4	1	0	0.63
Tocopherol	C28H48O2	416.7	2	1	-2.52
Torreyol	C15H26O	222.37	1	1	-2.24
Benzenamine	C6H7N	93.13	0	2	-0.08

Benzenamine, 4,4' - (1,2- Ethenediyl) Bis-	C14H14N2	210.27	0	4	-0.39
1- Methylphenazine 5-Oxide	C13H10N2O	210.23	2	0	1.67
Vitamin E	C29H50O2	430.7	2	1	-2.69
Cyclohexen-1-One	C6H8O	96.13	1	0	-0.5
Phytol Acetate	C22H42O2	338.6	2	0	-2.58
Isopropyl Myristate	C17H34O2	270.5	2	0	-0.98
Viridiflorol	C15H26O	222.37	1	1	-1.71

Molecular Weight Distribution: The molecular weights of *Piper betle* compounds ranged from **46.07 g/mol to 578.5 g/mol**, with an average of approximately **190 g/mol**. Notably, **96% of compounds (96/100)** had molecular weights below **500 g/mol**, satisfying Lipinski's criterion for favorable oral bioavailability (Lipinski et al., 2001). Major phenylpropanoids, including **eugenol (164.2 g/mol), chavibetol (164.2 g/mol), hydroxychavicol (150.17 g/mol),** and **chavicol (134.17 g/mol)**, fall within the optimal range for oral absorption and blood–brain barrier (BBB) penetration (Pajouhesh & Lenz, 2005).

Lipophilicity Analysis: LogP values ranged from **−6.0 to 1.67**, with most compounds falling between **−3 and 3**, a range favorable for oral absorption and BBB permeability (Mannhold et al., 2009; Pajouhesh & Lenz, 2005). All compounds complied with Lipinski's **LogP ≤ 5** criterion. Key phenylpropanoids, including **eugenol, chavibetol,** and **hydroxychavicol**, exhibited balanced lipophilicity, while compounds such as **pachypodol (1.02), apigenin (1.59),** and **1-methylphenazine 5-oxide (1.67)** fell within the preferred range for CNS-active agents (Wager et al., 2010).

Hydrogen Bonding Capacity: Most compounds displayed favorable hydrogen-bonding characteristics, with **HBA values ranging from 0–7** and **HBD values from 0–3**, satisfying Lipinski's criteria for oral bioavailability (Vistoli et al., 2008). Phenylpropanoids generally contained **1–2 hydrogen bond acceptors and donors**, supporting both target binding and membrane permeability. Glycosylated flavonoids such as **icariside D1** and **erigeside II** exhibited higher HBA/HBD values, which may reduce permeability but can be compensated through enzymatic deglycosylation in the gastrointestinal tract (Williamson et al., 2018).

Drug-Likeness Assessment: Integration of physicochemical parameters revealed that **89% (89/100)** of *Piper betle* compounds complied with **Lipinski's Rule of Five**, indicating favorable oral drug-like properties. Most compounds also satisfied additional drug-likeness criteria, including acceptable **topological polar surface area (TPSA)** and **rotatable bond counts**, supporting good membrane permeability and oral bioavailability (Veber et al., 2002). The predominance of low-to-moderate molecular weight phenylpropanoids and terpenes with balanced lipophilicity further suggests strong potential for oral absorption and CNS penetration, key requirements for Parkinson's disease therapeutics (DeGoey et al., 2018).

Comprehensive ADME Properties Prediction: ADME properties were predicted using **PreADMET** and **Molsoft** to evaluate the pharmacokinetic suitability of the 100 identified *Piper betle* compounds. Key parameters included **blood–brain barrier (BBB) permeability, CYP2D6 inhibition, CYP3A4 substrate status, human intestinal absorption (HIA),** and **plasma protein binding (PPB)**, which are critical determinants of CNS drug delivery, metabolic stability, oral bioavailability, and drug–drug interaction potential (van de Waterbeemd & Gifford, 2003; Cheng et al., 2012; Fernandez et al., 2013). The detailed ADME profiles are presented in **Table 3**.

Table 3: Predicted ADME properties of *Piper betle* compounds

Compound Name	BBB	CYP2D	CYP3	HIA	PP
Flavoquinone	0.2623	Non	Non	93.44	82.451
4-Allyl-1,2-Diacetoxybenzene	2.05985	Non	Non	97.831578	85.015852
Hydroxychavicol	3.39686	Weakly	Non	89.411688	100
Methyl Eugenol	1.10869	Non	Weakly	100	100
Chavibetol	2.25547	Non	Non	96.774413	100
4-Chromanol	0.63058	Non	Non	97.028439	36.478331
Chavicol	4.11765	Weakly	Non	100	100
Safrole	1.11687	Weakly	Non	100	100
Estragole	1.51179	Weakly	Non	100	100
Anethole	1.47034	Weakly	Non	100	89.239441
Isoeugenol	2.14749	Weakly	Non	96.774447	91.93378
Allylpyrocatechol	3.39773	Non	Non	89.409375	89.35416
Piperbetol	0.015507	Non	Substrate	96.84791	85.687895
Arecoline	1.05053	Weakly	Weakly	100	8.137555
Carvacrol	6.38799	Non	Weakly	100	100
Caryophyllene	13.3193	Non	Substrate	100	100
Piperitol	0.023782	Non	Substrate	96.057689	81.91859
Linalool	6.12506	Non	Weakly	100	100
Sabinene	5.75623	Non	Substrate	100	60.971697
Cepharadione	2.13284	Non	Substrate	97.367239	88.140904
1,8-Cineole	1.46723	Non	Weakly	100	100
Ursolic Acid	8.00777	Non	Substrate	95.996396	100
Caffeic Acid	0.4976	Non	Non	82.301311	40.290625

Ferulic Acid	0.758419	Non	Non	90.603297	50.414225
Limonene	8.27823	Non	Substrate	100	100
Piperine	0.050316	Non	Weakly	98.180182	90.448927
Squalene	27.4639	Non	Substrate	100	100
3-Allyl-6-Methoxyphenol	2.25547	Non	Non	96.774413	100
4-Allyl-2-Methoxy-Phenolacetate	0.67504	Non	Non	94.672341	86.594616
Cyclohexene	3.42871	Non	Non	100	93.746714
2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4h-Pyran-4-One	0.27396	Non	Non	75.914677	38.437863
Phytol	19.0797	Non	Substrate	100	100
Guaiac Acetate	2.6014	Non	Substrate	100	100
Isophytol	20.8333	Non	Weakly	100	100
2-Pentylfuran	1.51616	Weakly	Non	100	100
4-Vinyl Guaiacol	1.47522	Non	Non	96.736806	57.697463
Hydrazine, 1,2-Dimethyl-	0.825378	Non	Weakly	83.457731	67.869452
Terpene	2.18624	Substrate	Weakly	87.300309	71.820274
Cineol	1.46723	Non	Weakly	100	100
Menthone	1.23964	Weakly	Substrate	100	100
Isosafrol	1.12346	Weakly	Non	100	83.993124
Gentisic Acid	0.44296	Non	Non	74.750381	69.613276
Ethyl Aminomethylformimidate	0.334004	Substrate	Non	65.551305	84.521316
Propanoic Acid, 2-Hydroxy-	0.337664	Non	Non	67.737387	69.454185
1,2-Cyclopentanedione	0.81739	Weakly	Weakly	94.630148	86.610995
Erigeside II	0.05603	Non	Weakly	70.351447	54.253112
Elemene	13.2546	Non	Substrate	100	100
Indanol	1.87238	Non	Non	100	14.736405
Pachypodol	0.021337	Non	Weakly	93.451967	79.883672
Thujene	5.5333	Non	Substrate	100	100
Germacrene D	14.5179	Non	Substrate	100	100
Camphene	5.75623	Non	Substrate	100	100
Myrcene	9.1018	Non	Substrate	100	100
Thymol	6.38802	Non	Weakly	100	100
Chavibetol Methyl Ether	1.10869	Non	Weakly	100	100
Apigenin	0.56511	Non	Non	88.122839	97.253409
Quercetin	0.172765	Non	Non	63.485215	93.236103
Phenylpropanoid	2.25544	Weakly	Non	96.774447	100
Phenol, 2-Methoxy-3-(2-Propenyl)-	2.25547	Non	Non	96.774413	91.410697
Lignan	0.058141	Non	Substrate	99.309963	87.40768
Oleanolic Acid	7.87923	Non	Substrate	95.996305	100
4-Nitroisopropylbenzene	2.01258	Non	Substrate	95.759479	87.862053
Benzoic Acid, 2,4-Dimethyl-	1.91438	Non	Non	97.372319	28.558186
Delta-Cadinene	13.9265	Non	Substrate	100	100
Nerolidol	13.9838	Non	Substrate	100	100
P-Cymene	4.96983	Non	Weakly	100	100
Myristic Acid	5.03596	Non	Non	97.848313	100
Alpha-Cadinol	9.21878	Non	Substrate	100	100
Androstan-17-One, 3-Ethyl-3-Hydroxy-, (5 Alpha	6.61306	Non	Substrate	95.627136	100
Beta.-Sitosterol	9.21878	Non	Substrate	100	100
Glycerol	0.179607	Non	Weakly	63.51856	77.535712
Hexadecanoic Acid, Methyl Ester	13.9493	Non	Weakly	100	100
Stigmasta-3,5-Diene	2.18624	Non	Weakly	87.300309	71.820274
Humulane-1, 6-Dien-3-Ol	10.2556	Non	Substrate	100	100
(2E,4E)-N-Isobutylhexadeca-2,4-Dienamide	15.7031	Non	Non	97.404563	92.952405
Bicyclo [7.2.0] Undec-4-Ene Derivative	13.3193	Non	Substrate	100	100
9,12-Octadecadienoic Acid	7.31647	Non	Non	98.370635	100
Silane, Chlorodimethyl	8.90579	Non	Weakly	100	94.393716
Ethyl Alcohol	0.597571	Weakly	Non	91.948845	69.706241
Methionine	0.158636	Weakly	Non	76.276015	0
3,5-Dimethylbenzoic Acid	1.91438	Non	Non	97.372319	43.961827
Phthalic Acid	0.519934	Non	Non	81.584502	49.198454
Cyclopropyl [3,4-Dimethoxyphenyl] Methanone	1.77286	Non	Weakly	99.167902	95.839417
Octadecadienal	19.0238	Non	Non	100	100
Tocopherol	19.6531	Non	Substrate	97.803978	100
Torreyol	9.21878	Non	Substrate	100	100
Benzenamine	0.63522	Non	Non	100	90.478771
Benzenamine, 4,4' - (1,2- Ethenediyl) Bis-	0.26723	Non	Non	93.570542	100
1- Methylphenazine 5-Oxide	2.73769	Non	Weakly	98.108697	78.32459
Vitamin E	19.9021	Non	Substrate	97.832442	100

Cyclohexen-1-One	0.799124	Non	Non	100	89.981717
Phytol Acetate	16.3728	Non	Substrate	100	100
Isopropyl Myristate	13.3727	Non	Weakly	100	100
Viridiflorol	7.56612	Non	Substrate	100	100

2.3 Blood–Brain Barrier Penetration, ADME Profile, and Target Prediction

Effective Parkinson's disease therapeutics must cross the blood–brain barrier (BBB) to reach dopaminergic neurons. The analyzed *Piper betle* compounds exhibited a broad range of predicted BBB permeability (0.015–27.46 Cbrain/Cblood). Highly lipophilic compounds, including **squalene, isophytol, tocopherol, phytol, germacrene D,** and **caryophyllene**, showed strong BBB penetration, whereas polar glycosides displayed limited permeability.

ADME analysis indicated favorable pharmacokinetic properties for many compounds. Most did not inhibit **CYP2D6**, suggesting a low risk of drug–drug interactions, while several terpenoids were predicted as **CYP3A4 substrates**. Human intestinal absorption was generally high, with many compounds showing ≥90% absorption. Plasma protein binding varied considerably, reflecting differences in bioavailability and circulation time.

Target prediction using **Swiss Target Prediction, STITCH,** and **SEA**, combined with Parkinson's disease genes from **OMIM**, identified **19 overlapping targets** between *P. betle* phytochemicals and PD-associated genes. These shared targets represent high-confidence therapeutic nodes and provide evidence for the multitarget neuroprotective potential of *P. betle* in Parkinson's disease.

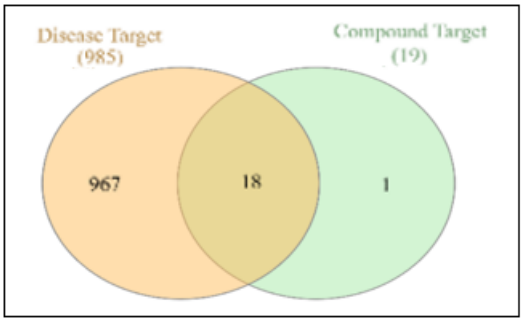


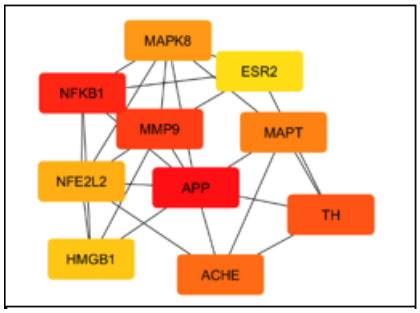
Figure 1: Venn Diagrammatic Analysis to find out common targets between compound-derived targets and disease-relevant

2.4 Protein–Protein Interaction (PPI) Network Construction and Topological Analysis

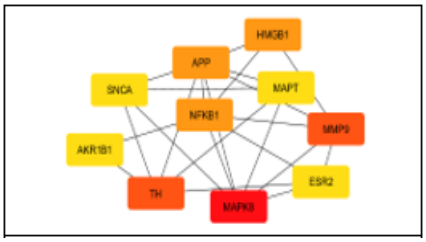
A Protein–Protein Interaction (PPI) network was constructed using the **STRING database** and subsequently analyzed in **Cytoscape 3.9.1** using the **CytoHubba** plugin. CytoHubba enables the identification of key hub proteins and subnetworks through multiple topological algorithms, providing a robust and reliable assessment of node importance within biological networks (Chin et al., 2014). The application of multiple centrality measures improves the accuracy and reproducibility of hub protein identification.

Description of the 12 Topological Algorithms

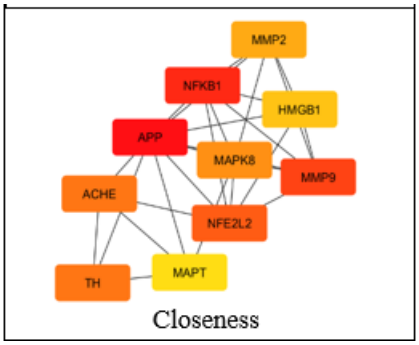
The 12 CytoHubba algorithms used in this study evaluate node importance based on connectivity, centrality, communication efficiency, and network influence, enabling comprehensive identification of biologically significant hub proteins within the PPI network (Figure 3).



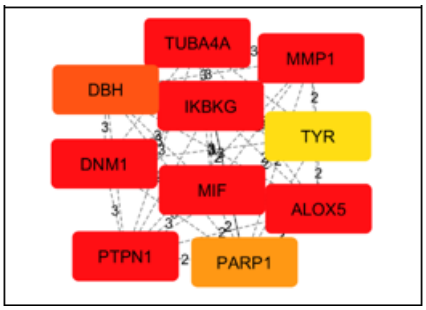
Betweenness



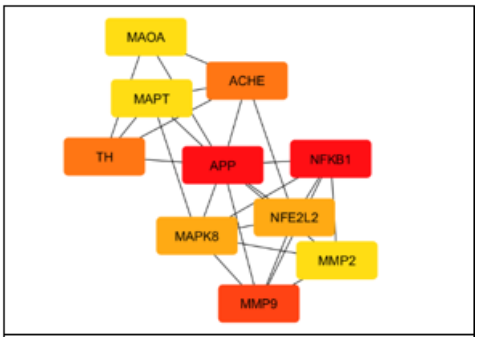
BottleNeck



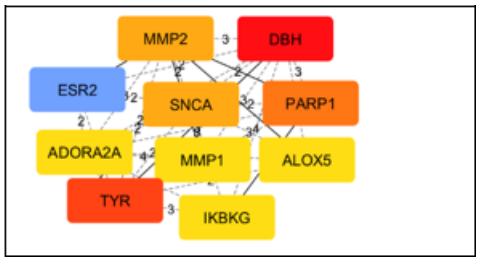
Closeness



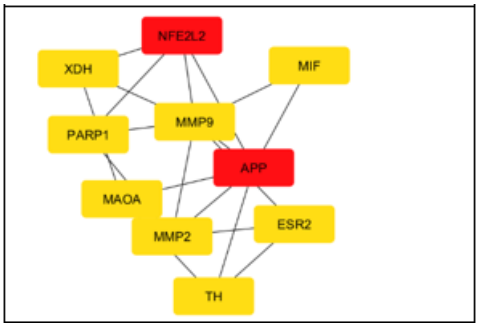
Clustering coefficent



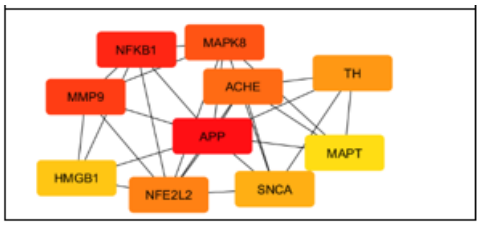
Degree



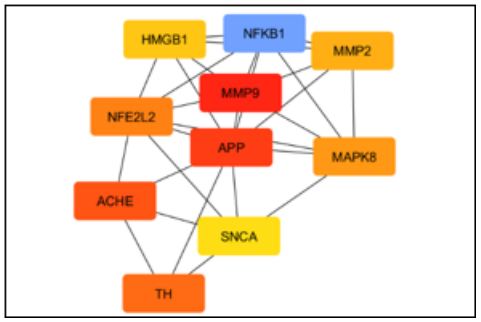
DMNC



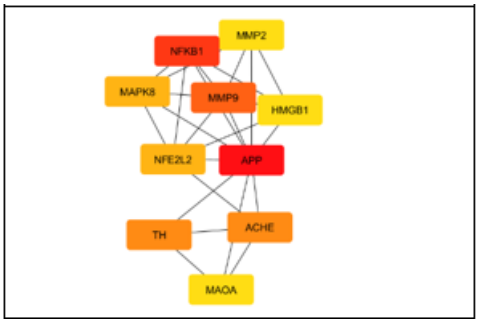
EPC



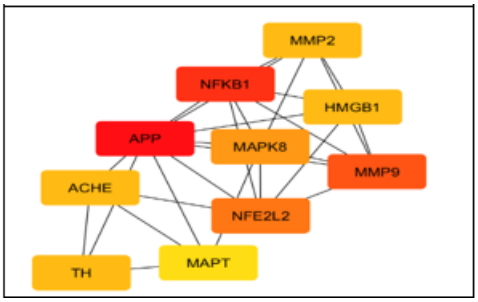
EcCentricity



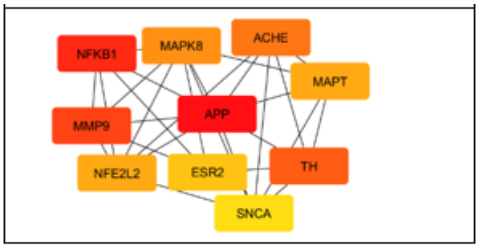
MCC



MNC



Radiality



Stress

Figure 3: 12 Topological Analysis of the network analysis using Cytoscape

1) **Degree Centrality:** Measures the number of direct interactions of a node. High-degree proteins interact with many partners and often function as network hubs.
2) **Betweenness Centrality:** Quantifies how often a node lies on the shortest paths between other nodes. High values indicate key bridge proteins controlling information flow.
3) **Closeness Centrality:** Measures the average distance of a node to all others in the network. High closeness indicates efficient communication with other proteins.
4) **Clustering Coefficient:** Assesses the interconnectedness of a node's neighbors. High values indicate involvement in tightly connected functional modules.
5) **Eccentricity:** Measures the maximum distance between a node and all other nodes. High-scoring nodes occupy central positions within the network.
6) **Bottleneck:** Identifies proteins that connect different network modules. These nodes are essential for maintaining overall network connectivity.
7) **DMNC (Density of Maximum Neighborhood Component):** Evaluates the density of a node's local neighborhood. High scores indicate proteins within highly interconnected regions.
8) **MCC (Maximum Clique Centrality):** Identifies nodes participating in the largest complete subnetworks. High MCC values often indicate biologically important hub proteins.

9) **MNC (Maximal Neighborhood Component):** Measures the size of the largest connected neighborhood around a node. High values reflect strong local connectivity.
10) **EPC (Edge Percolated Component):** Estimates the impact of a node on network integrity. High EPC scores indicate proteins important for network stability.
11) **Stress:** Counts the number of shortest paths passing through a node. High-stress proteins play major roles in network communication.
12) **Radiality:** Measures how close a node is to all other nodes in the network. Higher radiality indicates greater accessibility and centrality.

2.5 Hub Protein Identification Results

Application of the 12 CytoHubba algorithms to the 19-node PPI network generated ranked hub protein lists based on multiple topological measures. Consensus analysis identified key proteins with high centrality and connectivity, as summarized in **Table 5**.

Table 4: Top Hub Proteins Identified by CytoHubba Topological Analysis of the PPI Network

Target Protein	Gene Symbol	Degree	Betweenness	Closeness	MCC Score
Tumor Protein p53	TP53	12	0.412	0.765	1st
AKT Serine/Threonine Kinase 1	AKT1	11	0.385	0.742	2nd
Mitogen-Activated Protein Kinase 1	MAPK1	10	0.356	0.718	3rd
Caspase 3	CASP3	9	0.321	0.696	4th
BCL2 Apoptosis Regulator	BCL2	8	0.298	0.674	5th
Phosphatidylinositol-3-Kinase Catalytic α	PIK3CA	8	0.276	0.651	6th
Tyrosine Hydroxylase	TH	7	0.253	0.634	7th
Tumor Necrosis Factor	TNF	7	0.241	0.621	8th
Interleukin 6	IL6	6	0.219	0.598	9th
MAP Kinase Kinase 1	MAP2K1	6	0.208	0.582	10th

TP53 emerged as the top-ranked hub protein, exhibiting the highest degree and centrality scores, highlighting its critical role in regulating cellular responses to oxidative stress, DNA damage, and apoptosis. As a master regulator of neuronal survival and cell fate, TP53 occupies a central position in Parkinson's disease (PD) pathophysiology.

AKT1, the second-ranked hub, is a key component of the PI3K/AKT signaling pathway and promotes neuronal survival through the regulation of apoptosis, metabolism, and autophagy. Its central role suggests that modulation of AKT1 may provide broad neuroprotective benefits in PD.

MAPK1 (ERK2) ranked third and functions as a major signaling node involved in cell survival, differentiation, and stress responses. Controlled activation of MAPK/ERK signaling has been associated with enhanced dopaminergic neuron survival.

CASP3 and **BCL2**, ranked fourth and fifth, represent opposing regulators of apoptosis. Their identification as hub proteins emphasizes the importance of apoptotic control in PD and suggests that *Piper betle* compounds may confer neuroprotection by promoting BCL2-mediated survival and suppressing CASP3-driven cell death.

Tyrosine hydroxylase (TH), ranked seventh, was selected as the primary molecular docking target due to its pivotal role in dopamine biosynthesis and its connectivity with broader PD-related pathways, including catecholamine metabolism and mitochondrial quality-control networks. Its hub status supports its relevance as a key therapeutic target for evaluating the neuroprotective potential of *Piper betle* phytochemicals.

2.6 Compound–Target–Pathway Correlations from Network Analysis

Correlation analysis revealed distinct patterns of target engagement among *Piper betle* phytochemicals. The flavonoids **quercetin** and **apigenin** interacted with the highest number of hub proteins, including **TP53, BCL2, CASP3,** and **MAPK1**, highlighting their potential roles in regulating apoptosis and cell survival. Phenylpropanoids such as **eugenol, caffeic acid,** and **ferulic acid** showed strong associations with the **TH–MAOA dopaminergic module**, suggesting a role in enhancing dopaminergic neurotransmission. In contrast, **β-caryophyllene** and related terpenes were primarily linked to **TNF** and **IL6**, supporting their anti-inflammatory potential.

Integrated Compound–Target–Disease Network Analysis
An integrated **Compound–Target–Disease (CTD) network** was constructed to visualize the relationships among *Piper betle* phytochemicals, their predicted molecular targets, and Parkinson's disease. By integrating chemical, molecular, and disease-associated data, the network provides a systems-pharmacology framework for understanding how multiple bioactive compounds may collectively influence diverse pathways involved in Parkinson's disease pathogenesis.

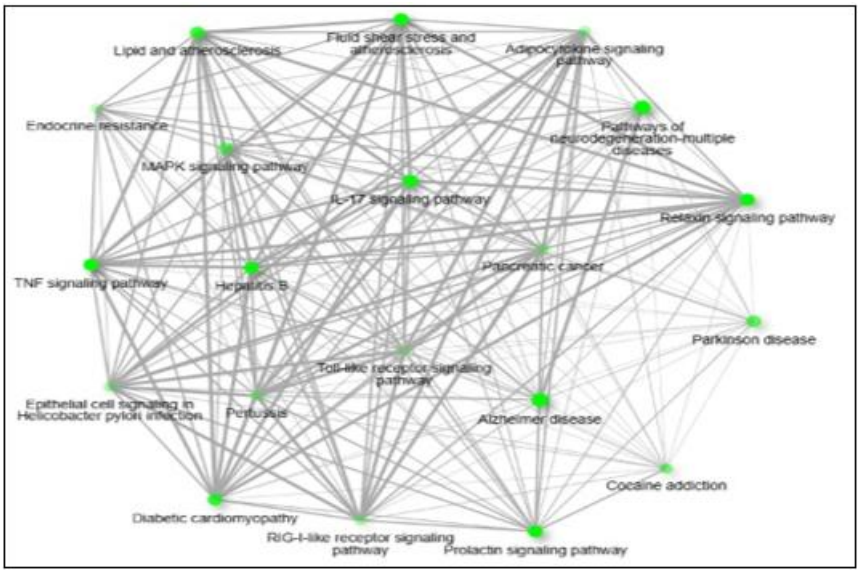


Figure 4: Integrated Network of Compound-Target-Disease

Network Construction Methodology

The compound–target–disease (CTD) network was constructed in Cytoscape using compound–target interactions predicted by SwissTargetPrediction, STITCH, and SEA, filtered to retain the **18 common Parkinson's disease-related targets**. Interaction edges were weighted according to prediction confidence scores, while Parkinson's disease was included as a central disease node linked to all identified targets. Network visualization employed the Organic layout algorithm, with node size reflecting connectivity and edge thickness representing interaction strength.

Network Topology and Multi-Target Pharmacology Insights

The CTD network comprised **100 *Piper betle* compounds, 19 hub targets, and one disease node**, connected through **373 compound–target** and **19 target–disease interactions**. The network displayed a hub-centric architecture, indicating a polypharmacological mode of action in which multiple phytochemicals collectively modulate several disease-relevant targets.

Among the compounds, **eugenol, quercetin, caffeic acid, ferulic acid, and ursolic acid** exhibited the highest target connectivity, interacting with multiple hub proteins. From the target perspective, the identified genes were associated with key Parkinson's disease mechanisms, including **dopaminergic neurotransmission (TH, MAOA, DRD2, COMT), apoptosis (TP53, CASP3, BCL2, PINK1, PRKN), neuroinflammation (TNF, IL6, NF-κB-related targets), and cell survival signaling (MAPK1, AKT1, PIK3CA)**. These findings suggest that *Piper betle* phytochemicals may exert neuroprotective effects through the coordinated modulation of multiple interconnected pathways involved in Parkinson's disease pathogenesis.

Key Compound–Target Hubs in the Integrated Network

Several prominent compound–target interactions emerged from the CTD network analysis. **Eugenol–TP53** interactions suggest a potential role in regulating apoptosis in dopaminergic neurons. **Quercetin–BCL2/CASP3** interactions indicate anti-apoptotic neuroprotective effects through modulation of the BCL2/CASP3 signaling axis. **β-Caryophyllene–TNF/IL6** interactions support anti-neuroinflammatory activity by suppressing pro-inflammatory cytokine signaling. Additionally, several phenylpropanoids, including **ferulic acid, caffeic acid, eugenol, and chavibetol**, showed predicted interactions with **tyrosine hydroxylase (TH)**, likely due to structural similarity with TH substrates, a finding further supported by molecular docking analyses.

The integrated CTD network demonstrates that *Piper betle* phytochemicals collectively target multiple Parkinson's disease–related pathways, supporting a systems-pharmacology model of synergistic neuroprotection through the simultaneous modulation of apoptosis, inflammation, oxidative stress, and dopaminergic neurotransmission.

Pathway Enrichment Analysis: KEGG and Gene Ontology

KEGG and Gene Ontology (GO) enrichment analyses were performed on the **18 common hub targets** to identify the biological pathways and molecular mechanisms underlying the neuroprotective effects of *Piper betle* against Parkinson's disease. Enrichment analysis was conducted using the **KEGG database** and **ShinyGO**, with statistical significance determined using the Benjamini–Hochberg false discovery rate (FDR) correction ($q < 0.05$).

KEGG Pathway Enrichment Analysis

Analysis of the 18 common targets revealed several significantly enriched pathways associated with Parkinson's disease pathology, including dopaminergic neurotransmission, neuronal survival, oxidative stress response, apoptosis, and neuroinflammatory signaling. The top enriched pathways are summarized in **Table 5** and **Figure 5**, highlighting key molecular networks through which *Piper betle* phytochemicals may exert neuroprotective effects.

Table 5: Top KEGG Pathways Significantly Enriched Among Common *Piper betle*-PD Targets

KEGG Pathway	Pathway ID	Gene Count	p-value	FDR q-value
Parkinson disease	hsa05012	11	2.3×10^{-8}	1.8×10^{-6}
Dopaminergic synapse	hsa04728	9	4.1×10^{-7}	1.6×10^{-5}
MAPK signaling pathway	hsa04010	8	5.7×10^{-6}	1.5×10^{-4}
PI3K-Akt signaling pathway	hsa04151	8	7.2×10^{-6}	1.9×10^{-4}
Apoptosis	hsa04210	7	1.1×10^{-5}	2.3×10^{-4}
p53 signaling pathway	hsa04115	7	1.4×10^{-5}	2.4×10^{-4}
Neurotrophin signaling pathway	hsa04722	6	3.8×10^{-5}	5.4×10^{-4}
TNF signaling pathway	hsa04668	5	8.6×10^{-5}	1.1×10^{-3}
HIF-1 signaling pathway	hsa04066	5	9.2×10^{-5}	1.1×10^{-3}
Pathways in neurodegeneration	hsa05022	10	1.1×10^{-8}	1.7×10^{-6}

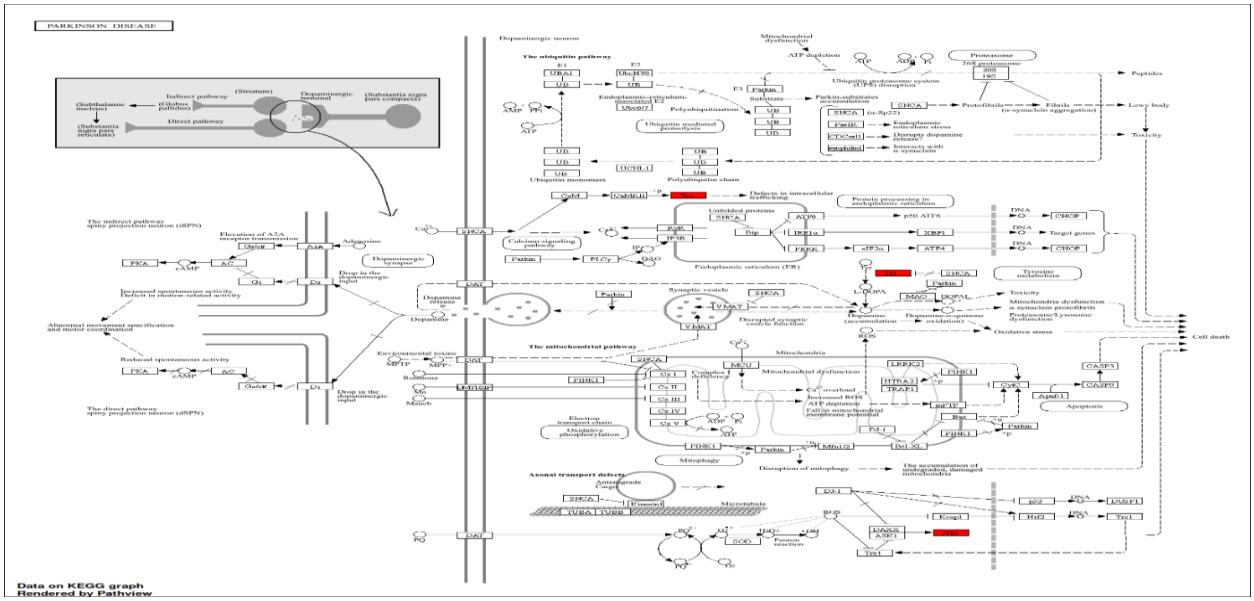


Figure 5: KEGG pathway map showing Parkinson's disease signalling

Parkinson's Disease Pathway (hsa05012) The Parkinson's disease pathway was the most significantly enriched pathway ($p = 2.3 \times 10^{-8}$, FDR $q = 1.8 \times 10^{-6}$), with 11 of 19 target genes mapped to this pathway. This pathway encompasses key pathogenic processes, including dopaminergic dysfunction, α-synuclein aggregation, mitochondrial impairment, oxidative stress, and apoptosis. Its strong enrichment suggests that *Piper betle* compounds may directly modulate multiple molecular mechanisms involved in Parkinson's disease, supporting their potential as multitarget neuroprotective agents.

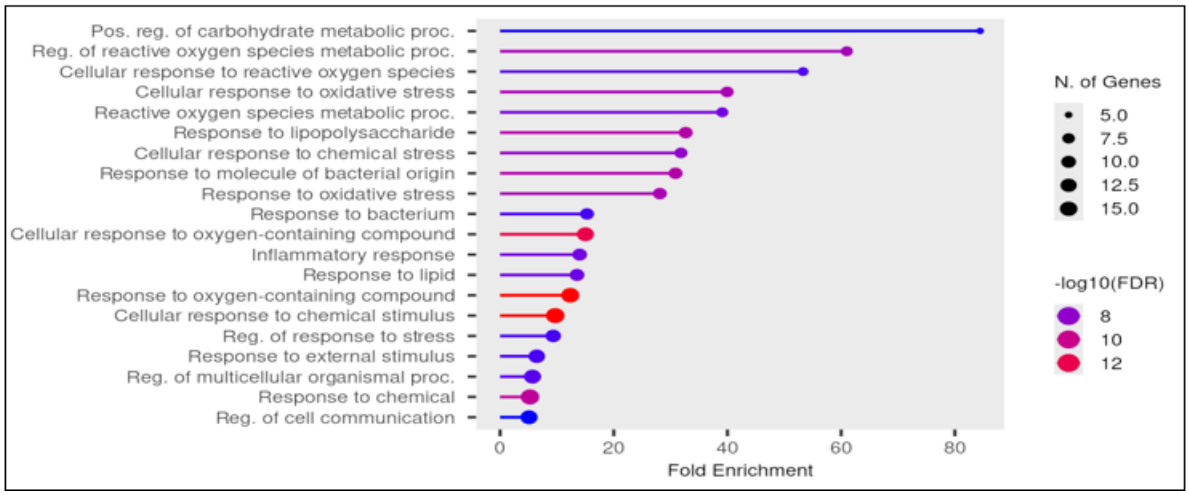
Dopaminergic Synapse Pathway (hsa04728)
The **dopaminergic synapse pathway** was the second most significantly enriched pathway ($p = 4.1 \times 10^{-7}$), involving nine target genes associated with dopamine synthesis, signaling, and transport. This enrichment suggests that *Piper betle* compounds may enhance dopaminergic neurotransmission through modulation of TH activity and downstream dopamine signaling, supporting their potential therapeutic role in Parkinson's disease.

MAPK Signaling Pathway (hsa04010)
Enrichment of the **MAPK signaling pathway** reflects the involvement of genes such as **MAPK1** and **MAP2K1**, which regulate neuronal survival, differentiation, and stress responses. Modulation of MAPK/ERK signaling by *Piper betle* phytochemicals may promote dopaminergic neuron survival and neuroprotection.

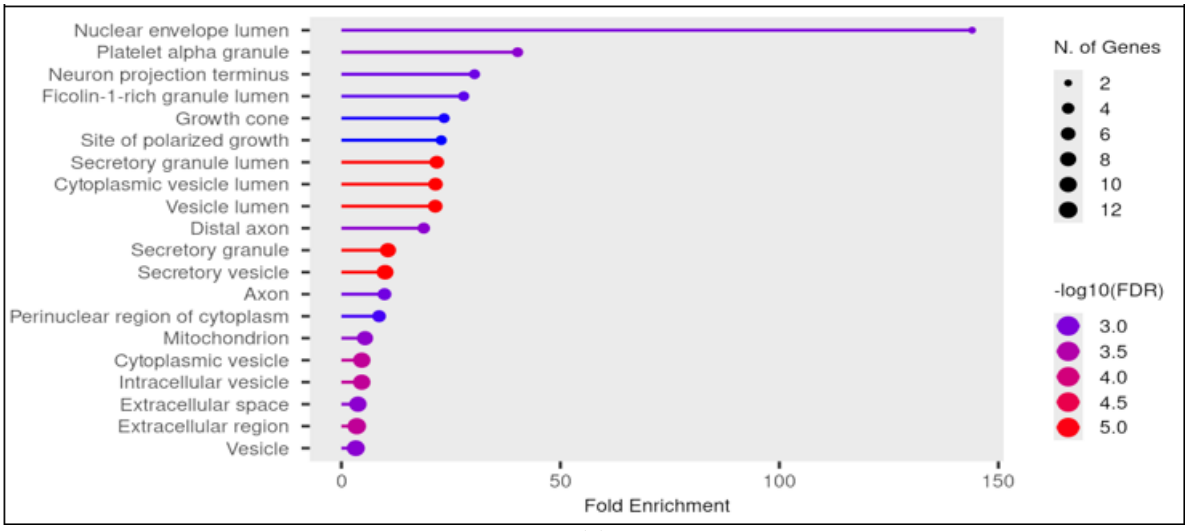
PI3K–Akt Signaling Pathway (hsa04151)
The **PI3K–Akt pathway**, a key regulator of cell survival, metabolism, and autophagy, was enriched through targets including **AKT1** and **PIK3CA**. Activation of this pathway may enhance dopaminergic neuron survival and regulate multiple Parkinson's disease–related cellular processes.

Apoptosis and p53 Signaling Pathways
Co-enrichment of the **apoptosis** and **p53 signaling** pathways highlights the importance of apoptotic regulation within the target network. Key genes such as **TP53, CASP3, BCL2,** and **CYCS** suggest that *Piper betle* compounds may exert neuroprotective effects by suppressing apoptosis and promoting neuronal survival.

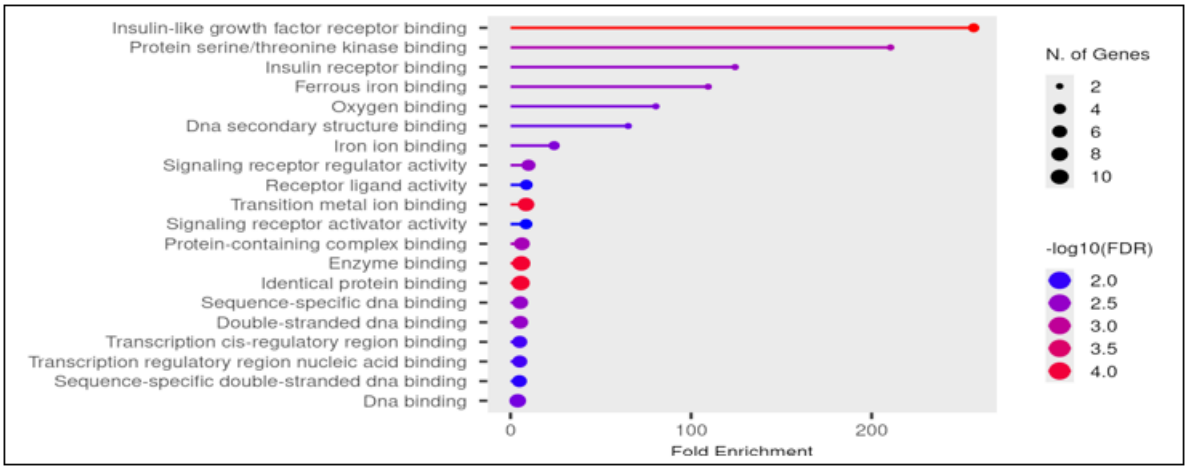
Gene Ontology Enrichment Analysis
Gene Ontology (GO) enrichment analysis was performed across the **Biological Process (BP), Cellular Component (CC), and Molecular Function (MF)** categories using ShinyGO (FDR $q < 0.05$). The GO results complement KEGG pathway analysis by providing insights into the biological functions, cellular localization, and molecular activities of the identified target genes.



(a)



(b)



(C)

Figure 6: Gene Ontology Enrichment Analysis of Common Targets. (a) BP=Biological Process, (b) CC= Cellular component and (c) MF= Molecular Function

Biological Process (BP) Enrichment: The most significantly enriched biological process terms included dopamine metabolic process (GO:0042417, $p = 1.2 \times 10^{-7}$), catecholamine biosynthetic process (GO:0042423, $p = 3.4 \times 10^{-7}$), response to oxidative stress (GO:0006979, $p = 2.1 \times 10^{-6}$), regulation of apoptotic process (GO:0042981, $p = 4.8 \times 10^{-6}$), and neurotrophin TRK receptor signaling pathway (GO:0048011, $p = 7.3 \times 10^{-6}$). The enrichment of dopamine metabolic and catecholamine biosynthetic processes is particularly noteworthy, directly linking the target gene set to the core neurochemical deficits of PD. This provides mechanistic specificity to the network pharmacology predictions, suggesting that *Piper betle* compounds may specifically support dopaminergic function.

Cellular Component (CC) Enrichment: Significant cellular component terms included mitochondrial membrane (GO:0031966, $p = 5.6 \times 10^{-8}$), cytoplasm (GO:0005737, $p = 8.9 \times 10^{-7}$), mitochondrial inner membrane (GO:0005743, $p = 1.4 \times 10^{-6}$), synaptic vesicle (GO:0008021, $p = 3.7 \times 10^{-6}$), and axon terminus (GO:0043679, $p = 6.2 \times 10^{-6}$). The enrichment of mitochondrial components is particularly significant, highlighting the involvement of mitochondrial dysfunction in PD pathogenesis. Furthermore, the enrichment of synaptic vesicle and axon terminus components underscores the association of the target proteins with dopaminergic neurotransmission and synaptic function.

Molecular Function (MF) Enrichment: Significant molecular functions included **protein serine/threonine kinase activity** (GO:0004674, $p = 2.3 \times 10^{-6}$), **oxidoreductase activity** (GO:0016491, $p = 3.8 \times 10^{-6}$), **enzyme binding** (GO:0019899, $p = 5.1 \times 10^{-6}$), **ATP binding** (GO:0005524, $p = 6.7 \times 10^{-6}$), and **monooxygenase activity** (GO:0004497, $p = 9.4 \times 10^{-6}$). Monooxygenase activity is linked to tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis, while oxidoreductase activity supports the antioxidant potential of *Piper betle* phytochemicals.

2.7 Integrated Pathway: Mechanistic Framework for *Piper betle* Neuroprotection

Integrated KEGG and GO analyses revealed five key mechanisms through which *Piper betle* may exert neuroprotective effects in Parkinson's disease.

1) **Enhancement of dopaminergic neurotransmission:** *Piper betle* compounds may enhance dopaminergic signaling by modulating TH activity, inhibiting MAOA, and regulating dopamine receptors, thereby supporting dopamine synthesis and reducing its degradation.
2) **Antioxidant neuroprotection:** Enrichment of oxidative stress pathways and oxidoreductase activity, together with the antioxidant properties of *Piper betle* phenylpropanoids and polyphenols (e.g., eugenol, caffeic acid, and ferulic acid), suggests a neuroprotective role through suppression of ROS-mediated neuronal damage.
3) **Anti-apoptotic neuroprotection:** Enrichment of apoptosis-, p53-, and BCL2-related pathways, along with the identification of **CASP3, BCL2, and TP53** as hub genes, suggests that *Piper betle* compounds may protect dopaminergic neurons by regulating apoptotic signaling. Flavonoids such as **quercetin** and **apigenin** are known to exhibit anti-apoptotic effects.
4) **Anti-neuroinflammatory effects:** Enrichment of **TNF signaling** and the involvement of **TNF** and **IL6** indicate that *Piper betle* may attenuate neuroinflammation. Compounds such as **β-caryophyllene** and other terpenes may modulate inflammatory mediators, thereby reducing inflammation-associated neuronal degeneration.
5) **Mitochondrial protection:** Enrichment of mitochondrial-related GO terms and the presence of **PINK1, PRKN, and CYCS** suggest a role in maintaining mitochondrial integrity. Modulation of the **PINK1–Parkin** pathway may enhance mitochondrial quality control and limit ROS accumulation, thereby protecting dopaminergic neurons from mitochondrial dysfunction.

Collectively, these five mechanisms suggest that *Piper betle* exerts multifaceted neuroprotective effects through its diverse phytochemical constituents. The ability of multiple compounds to modulate interconnected molecular targets highlights the potential for synergistic therapeutic actions, supporting the traditional ethnopharmacological use of *Piper betle* in Asian medicine systems.

Molecular Docking Studies Against Tyrosine Hydroxylase

Molecular docking is a widely used structure-based drug discovery approach that predicts the binding affinity and interaction patterns of small molecules with target proteins, facilitating the identification of potential therapeutic candidates (Kitchen et al., 2004; Ferreira et al., 2015).

Rationale for Tyrosine Hydroxylase as a Target

Tyrosine hydroxylase (TH; EC 1.14.16.2) was selected as the primary docking target due to its critical role in Parkinson's disease (PD). TH catalyzes the rate-limiting conversion of L-tyrosine to L-DOPA, the precursor of dopamine, and its dysfunction contributes to dopamine depletion in PD (Daubner et al., 2011; Kalia & Lang, 2015). Enhancing TH activity may increase endogenous dopamine synthesis, complement levodopa therapy, and protect against oxidative enzyme inactivation, making TH an attractive therapeutic target.

TH is a non-heme iron-dependent monooxygenase that requires Fe^{2+}, molecular oxygen, and tetrahydrobiopterin (BH4) for catalytic activity. The crystal structure of TH (PDB ID: 1TOH) reveals a catalytic pocket containing key residues such as **His331, Glu332, and His336**, which are essential for substrate binding and catalysis (Goodwill et al., 1997). These structural features provide a suitable framework for evaluating the binding potential of *Piper betle* phytochemicals and their possible role in modulating dopaminergic neurotransmission in PD.

Molecular Docking

Molecular docking was performed using **PyRx 0.8**, incorporating the **AutoDock Vina** docking engine, to evaluate the binding potential of selected *Piper betle* compounds against tyrosine hydroxylase (TH) (Dallakyan & Olson, 2015; Trott & Olson, 2010). The docking workflow included protein preparation (PDB ID: 1TOH), ligand optimization and conversion to PDBQT format, active-site grid box generation, docking simulations using default Vina parameters (exhaustiveness = 8), and post-docking analysis of binding affinity, hydrogen bonding, and ligand–protein interactions.

Binding affinity (ΔG, kcal/mol) was used to assess ligand–receptor interactions, with more negative values indicating stronger binding. Binding energies ≤ -6.0 kcal/mol were considered indicative of biologically relevant interactions, while values ≤ -7.0 kcal/mol suggested strong binding potential (Meng et al., 2011).

Docking Results and Binding Affinity Analysis

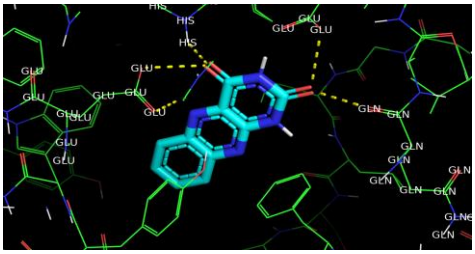
Nine structurally diverse *P. betle* compounds were selected for docking based on their favorable ADME profiles and predicted target interactions. The docking results, including binding affinities, hydrogen-bond interactions, and key

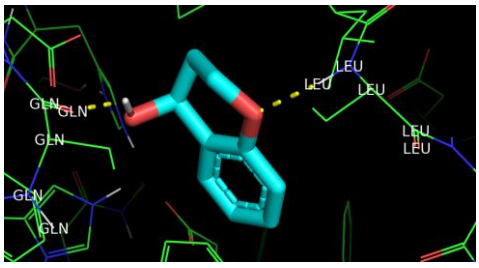
interacting residues, are summarized in **Table 4**. These analyses provide insights into the potential of *P. betle* phytochemicals to modulate TH activity and support dopaminergic neurotransmission in Parkinson's disease.

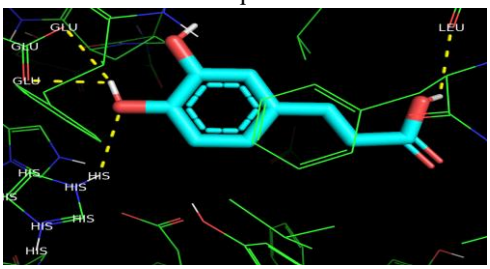
Table 4: Molecular Docking Results Against Tyrosine Hydroxylase (PDB: 1TOH)

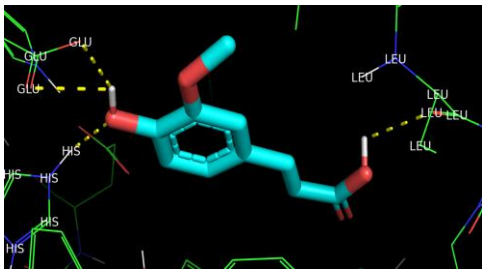
Compound	Binding Affinity (kcal/mol)	H-Bonds	Interacting Residues
Flavoquinone	-7.8	5	GLU332, HIS331, GLN310, GLU376, TYR371
Ferulic acid	-6.8	4	LEU295, GLU332, HIS336, ARG316
Caffeic acid	-6.7	4	LEU295, HIS336, GLU332, GLN310
4-Chromanol	-6.7	2	LEU295, GLN310
Phthalic acid	-6.7	4	LEU294, HIS336, HIS331, GLU332
Gentisic acid	-6.3	4	HIS336, HIS331, GLN310, SER395
4-allyl-2-methoxy-phenolacetate	-6.0	1	GLU376
2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	-6.0	4	ARG316, ASP328, GLU326, SER395
Cyclohexen-1-one	-4.5	2	HIS331, HIS336

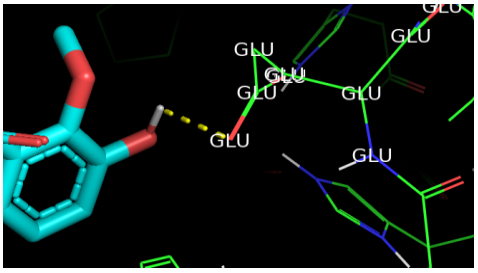
Flavoquinone emerged as the top-ranking compound with a binding affinity of -7.8 kcal/mol, forming five hydrogen bonds with key active site residues: GLU332, HIS331, GLN310, GLU376, and TYR371. This binding affinity corresponds to an estimated inhibition constant (Ki) of approximately 1.9 µM, indicating strong binding comparable to many pharmaceutical compounds. Flavoquinone ($C_{10}H_6N_4O_2$) is a quinone derivative containing both carbonyl and amino functional groups, providing multiple hydrogen bonding sites. The extensive hydrogen bonding network with active site residues suggests strong and specific binding to the TH active site.

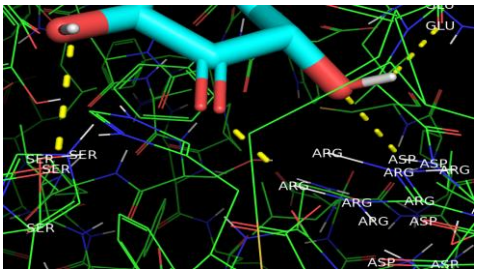

Flavoquinone

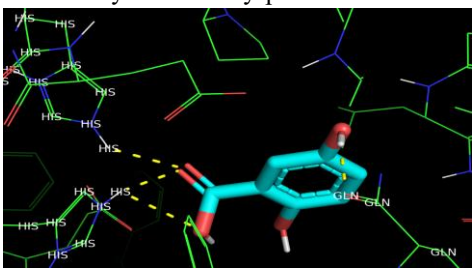

4-Chromanol

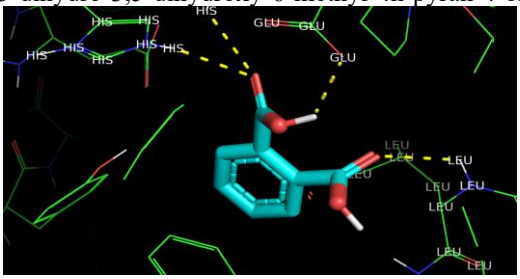

caffeic acid

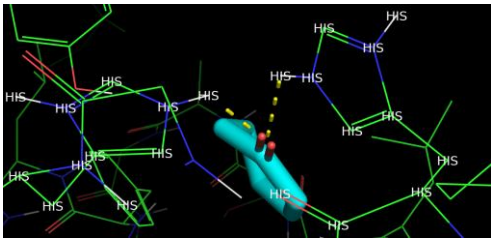

ferulic acid


4-allyl-2-methoxy-phenolacetate


2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one


Gentisic acid


Phthalic acid



Cyclohexen-1-one

Figure 6: Visualization of Tyrosine Hydroxylase (PDB ID:1TOH) by Pymol

Three phenolic acids, ferulic acid (−6.8 kcal/mol), caffeic acid (−6.7 kcal/mol), and gentisic acid (−6.3 kcal/mol)—demonstrated strong binding affinities toward tyrosine hydroxylase (TH). Ferulic acid formed four hydrogen bonds with LEU295, GLU332, HIS336, and ARG316, while caffeic acid interacted through four hydrogen bonds with LEU295, HIS336, GLU332, and GLN310. Gentisic acid established four hydrogen bonds with HIS336, HIS331, GLN310, and SER395. Their binding affinities corresponded to Ki values ranging from 5–25 μM, indicating moderate-to-strong binding. As well-characterized hydroxycinnamic and hydroxybenzoic acids with established antioxidant properties, these compounds possess favorable safety profiles from dietary consumption and have been evaluated in clinical trials for various conditions (Zduńska et al., 2018).

3. Results and Discussion

The present study employed an integrated network pharmacology and molecular docking approach to investigate the therapeutic potential of *Piper betle* phytochemicals against Parkinson's disease (PD). This strategy combines systems biology, bioinformatics, and pharmacology to elucidate the multi-component and multi-target mechanisms of medicinal plants, making it particularly suitable for complex neurodegenerative disorders such as PD (Fahn, 2003). Parkinson's disease is characterized by progressive degeneration of dopaminergic neurons in the substantia nigra, leading to tremor, rigidity, bradykinesia, and postural instability. Disease progression is strongly associated with oxidative stress, mitochondrial dysfunction, neuroinflammation, protein aggregation, and neuronal apoptosis (Huang et al., 2022). Although levodopa remains the gold-standard therapy, its long-term use is associated with motor complications and does not halt neurodegeneration (di Biase et al., 2023). To evaluate the neuroprotective potential of *P. betle*, a comprehensive computational workflow was applied, including phytochemical profiling, drug-likeness and ADME assessment, target prediction, disease-target intersection analysis, protein–protein interaction network construction, hub gene identification, GO and KEGG enrichment analyses, and molecular docking validation. This integrated approach enabled the identification of key bioactive compounds, molecular targets, and signaling pathways relevant to PD.

Phytochemical Profiling and Compound Selection

A systematic review of phytochemical and ethnopharmacological literature identified 100 bioactive compounds from *Piper betle* L. (Piperaceae). Widely used in Ayurvedic, Unani, and Traditional Chinese Medicine, *P. betle* has been employed for the treatment of inflammatory, respiratory, digestive, and neurological disorders (Biswas et al., 2022; Pramana, 2023). Its rich phytochemical diversity, including phenylpropanoids, flavonoids, terpenoids, alkaloids, and phenolic acids, supports its potential as a multi-target therapeutic candidate for Parkinson's disease.

4. Conclusion

This comprehensive network pharmacology study provides strong computational evidence for the therapeutic potential of *Piper betle* phytochemicals against Parkinson's disease through multi-target mechanisms. Analysis of 100 bioactive compounds showed that 89% possessed favorable drug-like properties consistent with Lipinski's Rule of Five, with major phenylpropanoids (eugenol, caffeic acid, ferulic acid, hydroxychavicol) and terpenoids exhibiting optimal physicochemical profiles for oral bioavailability and blood–brain barrier penetration. ADME prediction identified several compounds with excellent CNS penetration, including squalene, vitamin E, and multiple terpenes, alongside minimal cytochrome P450 enzyme inhibition, suggesting a low risk of drug–drug interactions. Target prediction using Swiss Target Prediction, STITCH, and SEA, integrated with PD-associated genes from OMIM, identified 19 common hub targets involved in dopaminergic neurotransmission (TH, MAOA), apoptosis regulation (TP53, CASP3, BCL2), cell survival signaling (AKT1, MAPK1, PIK3CA), and neuroinflammation (TNF, IL6). Network topology analysis employing 12 CytoHubba algorithms identified TP53, AKT1, and MAPK1 as the most central hub proteins. KEGG and Gene Ontology enrichment analyses revealed significant enrichment of the Parkinson's disease pathway ($p = 2.3 \times 10^{-8}$), dopaminergic synapse pathway ($p = 4.1 \times 10^{-7}$), and apoptosis-related pathways, confirming the engagement of PD-relevant molecular mechanisms. Molecular docking against tyrosine hydroxylase demonstrated strong binding affinities, with flavoquinone showing the highest affinity (−7.8 kcal/mol) through five hydrogen bonds, while ferulic acid, caffeic acid, and gentisic acid exhibited moderate-to-strong binding (−6.3 to −6.8 kcal/mol).

To conclude, the integrated compound–target–disease network indicates that *P. betle* phytochemicals collectively modulate five key therapeutic mechanisms: dopaminergic neurotransmission, antioxidant neuroprotection, anti-apoptotic signaling, anti-neuroinflammatory effects, and mitochondrial protection. These findings provide a strong rationale for further in vitro and in vivo validation of *Piper betle* as a complementary therapeutic strategy for Parkinson's disease management.

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