"REPURPOSING PHYTOCHEMICALS FOR LRRK2 G2019S AND α-SYNUCLEIN MODULATION IN PARKINSON'S DISEASE: A BIOINFORMATICS APPROACH"

Thesis submitted
in Partial Fulfilment of the Requirements for the
Degree of

MASTER OF SCIENCE

in

BIOTECHNOLOGY

by

Parneet Kaur
2K22/MSCBIO/36

Under the Supervision of
Prof. Pravir Kumar
Professor and Dean IA
Delhi Technological University

To the
Department of Biotechnology
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-110042, India

June, 2024

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CANDIDATE'S DECLARATION

I, **Parneet Kaur**, bearing Roll No. 2K22/MSCBIO/36 hereby certify that the work which is being presented in the thesis entitled "**Repurposing phytochemicals for LRRK2 G2019S and α-synuclein modulation in Parkinson's Disease: A Bioinformatics Approach**" in partial fulfilment of the requirement for the award of the Degree of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from January 2024 to May 2024 under the supervision of Prof. Pravir Kumar.

The matter presented in the thesis has not been submitted by me for the award of any degree of this or any other Institute.

PKaur

Candidate's Signature

This is to certify that the student has incorporated all the corrections suggested by the examiner in the thesis and the statement made by the candidate is correct to the best of our knowledge.

05/06/2024

Signature of Supervisor

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CERTIFICATE BY THE SUPERVISOR

Certified that **Parneet Kaur** (2K22/MSCBIO/36) has carried out their search work presented in this thesis entitled "**Repurposing phytochemicals for LRRK2 G2019S and α-synuclein modulation in Parkinson's Disease: A Bioinformatics Approach**" for the award of **Master of Science** from Department of Biotechnology, Delhi Technological University, Delhi under my supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the reward of any other degree to the candidate or to anybody else from this or any other University/Institution.

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Repurposing phytochemicals for LRRK2 G2019S and α-synuclein modulation in Parkinson's Disease: A Bioinformatics Approach

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ABSTRACT

Aim: Parkinson's disease represents a formidable global health challenge due to the deficiency of disease-modifying therapies, and the protracted usage of existing medications like Levodopa (L-dopa) is associated with incapacitating side effects. This investigation engrossed on employing an in-silico methodology to explore the drug-likeness and pharmacokinetics of select phytochemicals demonstrating notable permeability across the blood-brain barrier (BBB) and high gastrointestinal (GI) absorption, thereby identifying prospective therapeutic agents. Initially, a meticulously curated collection of 1,580 phytochemicals sourced from diverse medicinal plants underwent virtual screening for observance to Lipinski's Rule of Five applied using SwissADME, followed by scrutinizing binding interactions with Discovery Studio Visualizer, while blind docking simulations were executed employing PyRx, with docking parameters tailored to match the scoring system of a reference drug. Validation of the docking scores was accomplished using CB-Dock2. ADMET properties were prognosticated by means of SwissADME, and assessments of carcinogenicity and toxicity were conducted via CarcinoPred-EL and PkCSM tools, correspondingly.

Result: Five compounds, namely Isowithametelin, Yamogenin, Withametelin, Diosgenin, and Withametelin B, exhibiting promising pharmacological properties and low toxicity with high permeability across the BBB, were identified as potential inhibitors of LRRK2, a protein associated with Parkinson's disease. Additionally, two compounds, Withanolide A and Hederagenin, characterized by GI absorption, were also recognized as potential inhibitors of LRRK2 as they act in the gut inhibiting α-syn oligomerization and fibrillation. These compounds demonstrated either no or mild toxicity, indicating their potential for therapeutic application in Parkinson's disease.

Conclusion: Approximately of the phytochemical compounds identified in medicinal plants exhibit potential as drug candidates for treating Parkinson's disease caused by the mutant LRRK2 G2019S. This potential is attributed to their chronicled low binding energy and stability when interacting with the target protein.

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LIST OF ABBREVIATIONS

PD	Parkinson's disease
SNpc	substantia nigra pars compacta
LRRK2	Leucine-Rich Repeat Kinase 2
ROC	Ras of complex protein
LC	Locus Coeruleus
COR	C-terminal of ROC
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
GI	Gastrointestinal
BBB	Blood-Brain Barrier
IMPPAT 2.0	Indian Medicinal Plants, Phytochemistry And Therapeutics 2.0
LBs	Lewy bodies
α-syn	Alpha-synuclein
ANK	Ankyrin Repeat

CHAPTER 1

Introduction and Literature Review

1.1 Overview

Parkinson's disease (PD), initially outlined by James Parkinson in 1817, presents as a neurodegenerative condition characterised by four primary motor symptoms: bradykinesia, postural instability, resting tremor, and rigidity. It ranks as the second most predominant neurodegenerative disorder, marked through the specific loss of pigmented neurons in the substantia nigra pars compacta (SNpc) and the locus coeruleus (LC) within the brainstem. Key pathological features involve the deterioration of dopaminergic neurons in the SNpc and the development of Lewy bodies, which are α-synuclein-positive inclusions. Extensive neuropathological investigations indicate that PD encompasses degeneration across both the central and peripheral nervous systems, with the olfactory bulb and peripheral enteric nervous system being initial sites of involvement, followed by affliction of dopaminergic neurons in the SNpc during later disease stages. The clinical presentation of PD encompasses not only motor symptoms such as resting tremors, muscle stiffness, postural instability, and bradykinesia but also non-motor manifestations including disturbances in sleep, olfaction, urination, and bowel movements, with advanced stages associated with cognitive decline and hallucinations. Recent research has identified several proteins implicated in the progression of PD, including PTEN-induced putative kinase 1 (PINK1), α-synuclein (SNCA), VPS35, glucocerebrosidase (GBA), Parkin (PRKN), DJ-1, and leucine-rich repeat kinase 2 (LRRK2). While approximately 10% of PD cases result from inherited genetic mutations, the most common pathogenic mutation is the G2019S mutation in the LRRK2 protein. Furthermore, environmental factors such as exposure to toxins and lifestyle choices contribute to PD risk. Genome-wide association studies and meta-analyses have revealed a connection between LRRK2 polymorphisms and an increased likelihood of idiopathic PD. Among the roughly 18 familial PD-associated genes, those encoding LRRK2 and α-synuclein consistently correlate with idiopathic PD susceptibility.

1.2 Literature Review

1.2.1 Neurodegenerative Disease and PD

PD involves neurocyte degeneration in the substantia nigra, leading to striatal dopamine deficit and intracellular inclusions with α-synuclein aggregates. Diagnostic criteria for PD encompass neuromuscular impairments affecting the range and velocity of movement, along with manifestations such as rigidity and resting tremors. The molecular pathogenesis of PD encompasses a multitude of paths and procedures, comprising dysregulation of α-syn proteostasis, oxidative stress, impairment of mitochondrial function, perturbations in Ca^{2+} homeostasis, disruptions of then axonal transport pathway, and stimulation of neuroinflammatory processes [1].

Aggregates comprised of α-syn are universally detected within the neurons of individuals diagnosed with PD. Initially, soluble α-syn monomers undergo oligomerization, subsequently forming minor large insoluble fibrils of α-syn are neurotoxic and form cytoplasmic inclusions known as Lewy bodies (LB) [2]. The synergistic relationship between α-syn aggregation and mitochondrial dysfunction leads to PD pathology. Mitochondrial dysfunction may promote the α-syn aggregation, exacerbating the degeneration of neurons in PD patients [3]. Reduced mitochondrial complex 1 activity impacts electron transport, along with diminished levels of PPARγ co-activator 1α, are associated with mitochondrial dysfunction, leading to oxidative stress in Parkinson's disease [3], [4], [5]. Defects in DNA repair mechanisms can compromise the function of the dopaminergic axis, thereby elevating the susceptibility to PD [6]. Research has demonstrated a correlation between telomere shortening and PD pathogenesis [7]. Moreover, PD characterized by epigenetic alterations, including increased promoter methylation and histone modification changes [8]. Additionally, cells derived from PD patients exhibit a heightened rate of protein ubiquitination compared to those from healthy controls [9]. Mutations in genes such as PINK1 or PRKN (which encodes Parkin) are associated with PD. Research suggests that mitophagy, partially regulated by the PINK1-Parkin pathway, contributes to mitigating PD pathology. [10], [11]. Explorations into genetic and pharmacological modulation of NIX-dependent mitophagy present promising avenues for potential therapeutic interventions in PD [12]. In the realm of cellular senescence, the buildup of α-synuclein in Parkinson's disease (PD) is correlated with increased senescence, marked by an enhanced presence of senescent cells and brain tissue of PD patients exhibits elevated expression of senescence-associated β-galactosidase (SA-β-gal) [13]. During the early stages of PD, specifically stages 1–2 which represent presymptomatic phases, the pathological occurrence of inclusion bodies is predominantly localized within

specific brain regions, Medulla, pons, olfactory structures affected. The disease advances into stages 3–4, there is a transition in pathological engagement towards structures such as he substantia nigra and other midbrain and forebrain nuclei. Initially, these regions manifest mild changes, which subsequently progress to more pronounced alterations. At this juncture, most individuals likely transition into the characteristic phase of the disease. By stages 5–6, PD progresses to involve the mature neocortex, leading to a complete range of clinical PD symptoms [14].

1.2.2 The Structure and Localization of LRRK2

The LRRK2 gene encodes a substantial protein called dardarin, consisting of 2,527 amino acids, characterized by a complex structural configuration. This protein comprises fifty-one coding exonic regions and encompasses numerous functional domains, including multiple LRRs, a ROC domain coupled with its COR, a protein kinase domain, and a WD40 motif. The structural composition of the dardarin protein incorporates these distinct elements, illustrating its multi-domain architecture and emphasising the intricate molecular framework of LRRK2 [1]. The ARM, ANK, LRR, and WD40 domains facilitate numerous PPI, ROC-COR and kinase domains are vital for GTPase and kinase functions. This multifaceted arrangement renders LRRK2 a highly versatile and multifunctional protein. [15], [16]. LRRK2 is classified within the ROCO family, exhibiting notable sequence resemblance predominantly to its mammalian counterpart, LRRK1 [17]. Nevertheless, while sharing membership in the same family, LRRK2 and LRRK1 diverge notably in their kinase domains, indicating that they are not closely related homologs in this specific region of the protein [18]. LRRK2 exhibits widespread expression across various tissues, including the brain, heart, kidney, and lungs [19]. Additionally, LRRK2 has been identified in biofluids including urine, CSF, and blood, notably detected in PBMCs, encompassing lymphocytes and monocytes [20]. Within the cellular milieu, LRRK2 predominantly localizes in the cytoplasm, where it engages in phosphorylation-dependent interactions with the 14-3-3 adaptor protein [21], [22]. LRRK2 typically maintains an inactive state in the cytoplasm, facilitated by its association with the 14-3-3 adapter protein. Disruption prompts LRRK2 inclusion body formation, suggesting a role for 14-3-3 in stabilizing the proper folding of LRRK2 within the cytoplasm [21], [23].

The multidomain architecture of LRRK2 encompasses an ROC domain, spanning about 200 a.a. –250 a.a., trailed by a COR domain, spanning approximately 300 a.a. –400 a.a. The ROC region mediates LRRK2's GTPase activity, similar to the Ras superfamily, acting as a molecular switch modulating

kinase activity via guanine nucleotide binding. [24]. The structural analysis of LRRK2 indicates that its kinase domain spans amino acids 1878 to 2138, and is characterized as a serine/threonine kinase. Notably, the sequences of MLK1 and mitogen-activated protein kinase 9 (MKKK9) exhibit significant similarity to the kinase domain of LRRK2 [25]. Within the LRRK2 kinase domain, the active site is defined by specific structural elements, including a glycine-rich loop (G-loop, residues 1886–1893), a hinge region connecting the N- and C-terminal lobes (residues 1947–1951), the αC helix (residues 1915–1925), and the DYG motif (residues 2017–2019), which forms part of the activation loop (residues 2017–2042) [26]. By analyzing the sequence of the kinase domain, it has been determined that LRRK2 and LRRK1 exhibit the highest similarity with members of the RIP kinase family, [27], notably identified as RIP7 and RIP6, respectively. The RIP kinase family is crucial in extrinsic death, NF-κB signaling, and adaptive immune responses [28]. LRRK2 functions as a MAP kinase (MKKK), orchestrating the phosphorylation events of MKK4/7 and MKK3/6. This activates c-Jun N-terminal kinase and p38 MAPK pathways.. The intricately coordinated molecular cascade assumes a pivotal regulatory role in modulating cellular responses to stress stimuli [29].

1.2.3 LRRK2 and Its Mutations in PD

Around $\leq$ 5% of instances of sporadic PD and $\leq$13% of familial PD cases are attributed to specific genetic alterations, predominantly stemming from mutations in the LRRK2 gene. PD has been correlated with only a small subset of the over 100 identified mutations in LRRK2 thus far [30]. These genetic variations, located on the short arm (p arm) of chromosomes, constitute a subset contributing to the array of genetic variations linked with this neurodegenerative disorder [31]. Mutations in the LRRK2 gene follow an autosomal dominant pattern of inheritance, typified by stochastic penetrance, emphasizing the varied manifestation of phenotypic characteristics among affected individuals. The dominant genetic transmission can stem from either a "gain of function" phenomenon or haploinsufficiency-induced loss of function [32]. Seven missense mutations located within the LRRK2 gene have been identified as pathogenic. These mutations, namely R1441G, R1441C, R1441H, Y1699C, G2019S, R1628P, G2385R, and I2020T, are positioned in unique functional domains of the LRRK2 protein [33], [34].

Eight mutations, including I1371V, N1437H, R1441C/G/H, Y1699C, G2019S, and I2020T, are pathogenic in familial Parkinson's disease, with N1437D and R1441S seen in single families. These are associated with FPD as they occur at specific genetic loci known to be implicated in the pathogenesis of the disease.

1.3 Clinical Symptoms and Cerebral Pathology with LRRK2 Mutation

This section provides an overview of the clinical manifestations and neuropathological findings, where applicable, in individuals harbouring LRRK2 mutations.

I1371V Mutation: The I1371V mutation within the LRRK2 gene, discovered in a 2005 East Indian family (Family PD4) with overriding PD inheritance, manifests as typical PD symptoms at a relatively young age. Neuropathological analysis of a patient with this mutation revealed severe neuronal loss in the SNpc and moderate loss in the LC, alongside α-syn-positive Lewy pathology in affected brain regions and tau-positive neurofibrillary tangles in specific areas [35], [36]. Despite early onset and mild cognitive impairment, the observed neuropathology closely resembles typical PD, providing valuable insights into the clinical and pathological features associated with the I1371V LRRK2 mutation [37], [38].

N1437H Mutation: The N1437H mutation was recognized in a large Norwegian family (F04), spanning four generations, displaying autosomal dominant inheritance of familial parkinsonism. Clinical manifestations in affected individuals closely resembled sporadic PD. Neuropathological examination unveiled a substantial loss of melanin-containing neurons within the SNpc, alongside the presence of LBs in the remaining pigment-containing neurons. [39], [40]. Similar neuronal loss and α-syn pathology were observed in the LC and dorsal motor nucleus of the vagus. These findings suggest that the N1437H mutation leads to significant dopaminergic neuron loss and Lewy pathology in the midbrain, akin to typical PD [41].

N1437D Mutation: The N1437D mutation was identified in 2 Chinese families with FPD in 2020. Within these families, three affected individuals carried this mutation [42]. The average age of onset among these patients was 47.5 years, with two individuals showing onset at 44 and 51 years respectively, while onset age was not reported for one patient. The reported clinical symptoms of these patients were not detailed in the study. Additionally, neuropathological findings were not included in the report.

R1441C Mutation: The R1441C mutation is associated with the forfeiture of pigmented neurons in the SN and LC, accompanied by gliosis in affected individuals. LBs were detected in the remaining neurons within these regions. Clinically, the mutation leads to typical late-onset PD but exhibits diverse neurodegeneration patterns in the brainstem [43]. Identified in families across the USA, Brazil, Italy, Belgium, and China, individuals with the R1441C mutation typically present with symptoms resembling sporadic PD, with an average onset age of 60 years [44]. The mutation's occurrence across diverse ancestral lineages suggests the Arg1441 residue is a mutation hotspot.

R1441G Mutation: PD patients with the R1441G mutation typically exhibit an onset age of around 65 years, with levodopa-responsive parkinsonism and no cognitive impairment. Their motor symptoms closely resemble idiopathic PD, with fewer instances of autonomic dysfunction and less severe sympathetic denervation compared to sporadic PD [45]. Moreover, R1441G-associated PD patients demonstrate lower rates of cognitive and neuropsychiatric impairment and less hyposmia. Sibling variation in onset age suggests additional genetic or environmental factors at play. Neuropathological studies indicate approximately 60% loss of pigmented neurons in the substantia nigra, with no deposition of α-synuclein or Tau observed [46].

R1441H Mutation: Numerous global reports provide strong evidence for the pathogenicity of the R1441H mutation in familial cases, often with diverse haplotypes, underscoring its widespread prevalence. Onset age ranges from the 40s to 60s, with a clinical progression resembling sporadic PD. Neuropathological examination unveiled a significant decline in dopaminergic neurons and the presence of astrogliosis in the SNpc, with no evident impact on the LC. The lack of Lewy pathology in any cerebral region suggests exclusive degeneration of the nigral region without the deposition of α-syn or Tau proteins [43].

R1441S Mutation: Initial clinical manifestations included an asymmetric resting tremor that responded favorably to anti-parkinsonian therapy. Mild cognitive impairment either preceded or coincided with the onset of motor symptoms. Despite the presence of only one reported familial case, the pathogenic nature of the R1441S mutation is substantiated by its association with three other pathogenic mutations (R1441C, R1441G, and R1441H) at the same residue and its strong cosegregation with the disease phenotype [47].

Y1699C Mutation: The Y1699C mutation in LRRK2, associated with PARK8, shows statistical evidence of pathogenicity. Nonetheless, cases also exhibit amyloid plaques suggestive of mild to moderate AD and mild neurodegeneration consistent with motor neuron disease. Surviving neurons in the SN and LC contain abundant eosinophilic granules. Neuropathological analysis indicates LB pathology in the SN, LC, and olfactory bulb, with some LB present in the neocortex. Neurofibrillary tangles shown: hippocampus, entorhinal cortex, indicating diverse neuropathology associated with the Y1699C mutation [48], [49], [50].

G2019S Mutation: Patients with the G2019S mutation commonly exhibit typical PD symptoms, with longer disease duration but milder symptoms, implying slower disease progression. Histopathological analysis of three cases revealed neuronal loss and Lewy pathology in the SN [51], [52], [53]. Patients with the G2019S mutation typically experience symptom onset at a mean age of 57.4 years, resembling typical PD symptoms, and treatment-related dyskinesia. The pathogenicity of this mutation may be influenced by genetic and environmental factors beyond the LRRK2 mutation itself. Lewy pathology was consistently found in the brainstem across all cases, with occasional instances in the neocortex [54], [55], [56].

I2020T Mutation: Individuals with the I2020T mutation exhibit Mild to moderate SN pigmented neuron loss, unaffected LC [57]. LB abnormality was absent throughout the brain . Clinical features closely resembled typical idiopathic PD, with milder autonomic symptoms and preserved cognitive function. The rarity of the I2020T mutation is underscored by its limited identification in only two founders and sparse findings in case-control studies [58], [59]. Remarkably, pronounced neuronal loss in the substantia nigra pars reticulata, atypical for sporadic PD, was noted, alongside the lack of Lewy pathology. In one instance, neuropathological diagnosis indicated multiple system atrophy with parkinsonism (MSA-P), attributed to GCIs in the putamen. Overall, neuropathological variability associated with the I2020T mutation appears comparable to, or possibly more prominent than, that observed with the G2019S mutation [60], [61], [62].

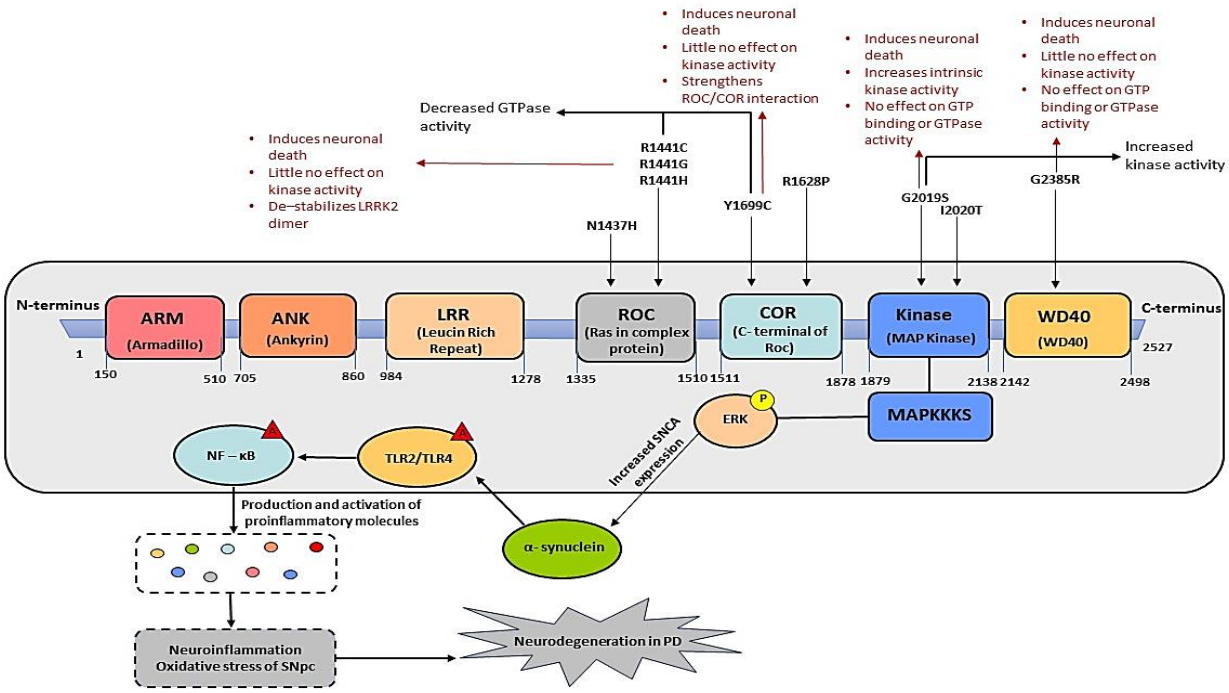

Fig 1.1: Diagram illustrating LRRK2's functional domains, pathogenic mutations, and effects

1.4 The Impact of Morbific Mutations on the Functionality and Molecular Characteristics of LRRK2

LRRK2 possesses both a ROC domain and a Ser/Thr protein kinase domain within a single polypeptide. Following the ROC domain is the carboxy-terminal of the ROC (COR) domain, the function of which remains uncertain [63], [64]. The ROC-COR tandem structure is shared among the ROCO protein family, indicating the probable significance of the COR domain in ROC functionality [65]. The pathogenic mutations mentioned are situated within the ROC-COR-kinase domains, suggesting their potential role in Parkinson's disease pathogenesis through modulation of these domains' functions and/or molecular properties.

1.4.1 Kinase Activity In Vitro and Phosphorylation Activity of Cellular Substrates

RRK2 is known to phosphorylate various proteins, including MBP [66], ERM family proteins [67], 4E-BP1 [68], ribosomal proteins s11/s15/s27 [69], p62/SQSTM1 [70], and small GTPase Rab proteins [71]. Additionally, LRRK2 undergoes autophosphorylation, particularly around the ROC domain [72], [73],

[74], [75]. In vitro, kinase assays using peptide substrates such as LRRKtide and Nictide have remained established for the enumeration of kinase activity [67], [76]. The G2019S mutation markedly boosts substrate and autophosphorylation compared to R1441C. Effects of other mutations on kinase activity vary between reports, possibly due to assay system differences. Autophosphorylation at Ser1292 is considered physiologically relevant, with all mutations except Y1699C increasing Ser1292 autophosphorylation [74]. Steger et al. identified Rab proteins as physiological substrates of LRRK2, with the G2019S mutation increasing Rab8A phosphorylation in vitro [71]. Overexpression of LRRK2 and substrate Rab proteins in cells, along with knock-in R1441G or G2019S mutations in mice, demonstrated enhanced Rab phosphorylation by all pathogenic mutations. Kalogeropulou et al. also reported increased cellular phosphorylation of Rab10 Thr73 by all pathogenic mutations [77]. Non-G2019S mutations did not alter Rab8A phosphorylation in vitro, it markedly increased in vivo, possibly due to the lipid-modified state of Rab proteins in cellular membranes. This suggests a role for subcellular localization in substrate phosphorylation by non-G2019S mutations [43].

1.4.2 LRRK2 GTPase Activity In Vitro

The ROC domain of LRRK2 if it is or not mutagenic has GTP and GDP binding capabilities, and exhibits GTPase action in vitro. Investigations into the impact of pathogenic mutations on this activity have yielded varied results [64]. While some studies observed upregulation of GTP-binding activity by certain mutations like I1371V, R1441C/G, and Y1699C, others found no significant changes or even attenuation, suggesting a degree of variability influenced by experimental conditions. The interface of the ROC and COR domains situated, these mutations have the potential to influence their interaction.. Regarding GTPase activity, mutations like R1441C/G and Y1699C have been shown to reduction in vitro GTPase activity, likely contributing to increased GTP-bound LRRK2 levels [63], [73]. However, inconsistencies exist in these findings across different studies. Structural alterations induced by ROC mutations might impact LRRK2's biological properties, including subcellular localization and interactions with substrate proteins like Rab. Nonetheless, how these changes precisely modulate LRRK2's kinase activity and substrate phosphorylation remains elusive. Further investigation into the structural and functional consequences of ROC mutations is crucial for elucidating their role in LRRK2-mediated signaling pathways [78], [79], [80], [81].

1.4.3 3D Structure

The ROC domain of LRRK2 interacts with its kinase domain, crucial for its kinase activity, implying regulatory roles. Recent cryo-EM analysis elucidated the full-length LRRK2 structure, revealing interdomain interactions between ROC, COR, ANK/LRR, and kinase domains [82]. Notably, FPD-linked mutations (Asn1437, Arg1441, Tyr1699) are situated in the ROC-COR interface, suggesting their significance in substrate phosphorylation. Another study proposed an active conformation for LRRK2, indicating mutations Asn1437 and Arg1441 may favor this state, while Tyr1699's substitution could facilitate conformational transitions. Ile2020's mutation stabilizes the active state by altering its environment [83]. The G2019S mutation may induce contained conformational changes within the kinase domain, possibly boosting kinase activity. Overall, non-G2019S pathogenic mutations potentially enhance substrate phosphorylation by modulating domain structure and regulating the transition between inactive and active states.

1.4.4 LRRK2 Phosphorylation Dynamics and Its Interaction with 14-3-3

Regulatory phosphorylation of LRRK2 at Ser910 and Ser935 is a consistent observation, yet the kinases responsible remain unknown [63]. Remarkably, LRRK2 inhibitors lead to dephosphorylation at these sites, though the underlying mechanisms remain elusive [84], [85]. These phosphosites serve as proxies for evaluating LRRK2 inhibitor efficacy in animal and human studies. Pathogenic mutations in LRRK2 distinctly regulate Ser910/935 phosphorylation, with the R1441C/G/H, Y1699C, and I2020T mutations suppressing phosphorylation, contrasting the G2019S mutation [86]. Additional phosphosites, such as Ser955 and Ser973, undergo similar regulation by pathogenic mutations. Phosphorylation at Ser910/935 promotes LRRK2's interaction with 14-3-3 proteins, but the functional implications remain unclear. Disruption of this interaction may affect LRRK2's subcellular localization, potentially resulting in the formation of filamentous structures in the cytoplasm. [86]. Notably, pathogenic mutations associated with filament formation exhibit reduced phosphorylation at Ser910/935 related to wild type and G2019S LRRK2. Recent cryo-electron microscopy studies have revealed the 3D structure of LRRK2 filaments around microtubules, suggesting a potential role in motor protein inhibition [87]. Further investigations are warranted to explicate how morbific LRRK2 mutations impact its interaction with microtubules and subsequent effects on motor protein dynamics.

1.4.5 Interactions with Additional Binding Partners

Besides 14-3-3 proteins, LRRK2 interacts with various other proteins in cultured cells, including FADD [88], DVL1–3 [89], MKK6/7 [90], Rac1 [91], Akt1 [92], α-tubulin [93], PP1 [94], and PKARII [95]. The effect of infectious LRRK2 mutations on these interactions varies, with unclear pathological relevance. Further studies needed to clarify LRRK2's impact on Rab phosphorylation. Additionally, the Rab32 subfamily, including Rab29, Rab32, and Rab38, represents significant binding partners of LRRK2 [96]. Rab29 acts as an activator of LRRK2 [97], [98] binding to LRRK2's ANK or ARM (1-552 a.a.) remains debated. Pathogenic LRRK2 mutations may ramblingly augment Rab29 binding to the amino-terminal part of LRRK2, potentially accelerating substrate Rab protein phosphorylation. Notably, despite mutations, Rab29 retains its ability to activate LRRK2 G2019S mutants, indicating that mutations do not transform LRRK2 into an incessantly stimulated form [97], [98].

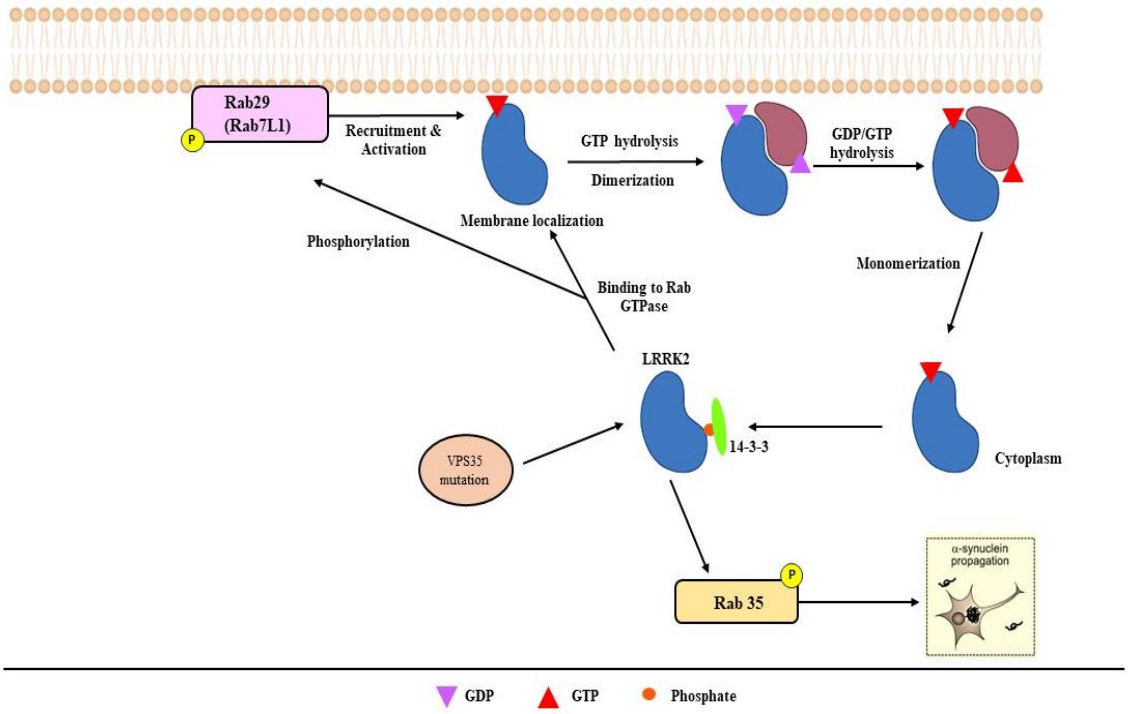

Fig 1.2: Activation cycle of LRRK2 and its association with other binding factors

1.5 Cellular Roles and Disease Mechanisms of LRRK2

The exact function of LRRK2 remains unclear, studies on Parkinson's disease-related mutant forms of LRRK2 and knockout animal models suggest its participation Roles include neurite outgrowth, cytoskeleton maintenance, vesicle trafficking, autophagy, and immune response, which underscores its significance. With numerous enzymatic domains and sites for protein interactions, LRRK2 is presumably involved in multiple cellular functions concurrently.

1.5.1 LRRK2 Kinase Activity

Since its discovery and characterization in 2004, extensive research has focused on understanding the kinase activity of LRRK2, particularly concerning its association with PD-linked mutations. Assessing the intrinsic kinase activity of LRRK2 typically involves measuring its autophosphorylation in vitro after purification from cultured cells or various tissues [99], [100]. Although LRRK2 exhibits self-phosphorylation, the physiological relevance of this autophosphorylation is uncertain, with only Ser^{1292} autophosphorylation detected in vivo, among various potential autophosphorylation sites identified. [101]. Remarkably, the G2019S mutation consistently displays heightened autophosphorylation at this site, correlating with enhanced neuronal survival. Despite thorough investigation, only a limited quantity of endogenous substrate of LRRK2 G2019S kinase activity has been definitively identified. Several proteins have been reported as potential substrates in in vitro settings, with implications for cytoskeletal dynamics and neuronal function. For instance, moesin [102]. Other potential substrates include β-tubulin [103], [104] and tau [105], with elevated phosphorylation observed in disease mutants, particularly G2019S and I2020T. These findings are supported by observations of aberrant neurites and disrupted microtubule networks in models expressing mutant LRRK2. Moreover, LRRK2's kinase activity appears to be significantly elevated in the brain compared to peripheral tissues, suggesting tissue-specific regulation mechanisms. While the phosphorylation of proteins like 4E-BP1 [106] and FoxO1 [107] by LRRK2 has been proposed, but their physiological relevance remains uncertain. Additionally, mutant LRRK2 has been implicated in the phosphorylation of MKK4 and activation of downstream pathways involved in apoptosis and autophagy regulation. These findings underscore the complex role of LRRK2 in cellular signaling pathways and the need for further research to elucidate its specific substrates and their implications in PD pathogenesis [107], [108], [109].

1.5.2 GTPase Activity of G2019S LRRK2

In additive to its kinase activity, LRRK2 features a GTPase function, a fundamental enzymatic activity vital for its cellular function. LRRK2's GTPase domain, part of the Roco protein superfamily, comprises an ROC domain followed by a downstream COR domain. The ROC domain of LRRK2 demonstrates comparable affinity for both GTP and GDP, a trait unaffected by PD-linked mutations, except for disruptions stemming from functional mutations in conserved P-loop residues [110], [111], [112], [113]. Kinase-dead mutations preserve GTP binding, while disease mutations, particularly in ROC and COR domains, impair GTP hydrolysis. Unlike kinase domain mutants, ROC and COR domain mutants exhibit reduced GTPase activity, hinting at a potential linkage between LRRK2's GTPase and kinase functions. Although the ROC and kinase domains of LRRK2 have a weak interaction, kinase activity hindered: the absenteeism of a GDP/GTP binding-competent ROC domain, likely due to disrupted dimerization [114], [115]. Autophosphorylation within the ROC domain may impede GTP binding, although further investigation is needed to elucidate its cellular implications. Recent studies have identified ARFGAP1 as a putative LRRK2 GTPase activator, phosphorylated by LRRK2 to enhance GTP hydrolysis. Intriguingly, ARFGAP1 localizes to the N-terminal region of LRRK2, suggesting an unconventional regulatory mechanism. Knockdown of ARFGAP1 ameliorated the neurite-shortening phenotype induced by mutant LRRK2, implicating its potential role in modulating LRRK2's neurotoxic properties [116], [117]. However, additional research is required to copiously elucidate the mechanism of ARFGAP1-mediated regulation of LRRK2 GTPase activity.

1.5.3 LRRK2 Oligomerization: Protein Assembly and Function

With few exclusions, it is widely acknowledged that LRRK2 primarily occurs in a dimeric state. Various experiments have demonstrated the presence of dimeric LRRK2 proteins, including co-immunoprecipitation studies where differently tagged LRRK2 proteins, including wild type and PD-mutant forms, interacted with each other. Electron microscopy analyses of purified LRRK2 proteins also revealed the presence of dimeric complexes [118]. Recent imaging techniques have shown both monomeric and oligomeric forms of LRRK2, with membrane-bound oligomeric LRRK2 observed near the cell periphery and in the plasma membrane [119]. However, there is some discrepancy regarding the interpretation of LRRK2's oligomeric state. While some studies suggest that LRRK2 predominantly exists as a dimer, others propose that LRRK2 may form higher-order oligomeric structures. The estimation of LRRK2's oligomeric state is challenging due to variations in experimental conditions and techniques used for analysis. Additionally, different cell types and extraction protocols may

influence the observed conformation of LRRK2. Recent studies utilizing innovative techniques have elucidated the oligomerization of LRRK2, especially in disease-linked mutations. These investigations reveal that mutations associated with disease in LRRK2 prompt a transition towards high-molecular-weight protein complexes. These mutant variants induce the formation of cytoplasmic filaments resembling structures associated with cell death pathways. Intriguingly, pharmacological inhibition of LRRK2 kinase activity similarly triggers the formation of filamentous structures, implying a connection between LRRK2 oligomerization and its neurotoxicity [120], [121]. Comprehending the interplay among LRRK2 oligomerization, kinase activity, and neurodegeneration is essential for unraveling the etiology of LRRK2-associated PD. Further exploration of the conformational alterations of mutant LRRK2 in pertinent in vivo models of neurodegeneration will offer valuable insights into the pathological mechanisms driving LRRK2-PD [122], [123].

1.5.4 LRRK2 Regulation of Autophagy and Lysosomal Function

Both wild-type and disease-mutant forms of LRRK2 seem to affect the autophagy/lysosomal protein degradation pathway. Overexpression of the G2019S mutant LRRK2 in differentiated human neuroblastoma cells leads to the accumulation of autophagic vacuoles in both soma and neurites, indicating a disturbance in basal autophagic activity. This effect is dependent on LRRK2 kinase activity and the presence of autophagy-related proteins LC3 or Atg7 [124], indicating the importance of LRRK2 in maintaining neurite integrity under normal and pathological conditions. Alegre-Abarrategui et al. [125] developed an in vitro expression system using R1441C mutant LRRK2, which similarly led to autophagic vacuole accumulation, signifying a probable site of LRRK2-mediated autophagy directive. Knockdown of LRRK2 under nutrient-rich circumstances increases LC3-II levels but prevents LC3-I to LC3-II maturation during starvation, indicating altered autophagy activity upon LRRK2 down-regulation. In vivo, transgenic models expressing G2019S and R1441C mutant LRRK2 display age-related neurite complexity reduction and autophagic vacuole accumulation [126]. Mutant LRRK2-induced disruption of autophagy may involve altered ER-dependent calcium homeostasis, leading to impaired lysosomal acidification. Wild-type LRRK2 also influences autophagic activity, suggesting a complex role for LRRK2 in modulating cellular autophagy flux [127].

1.5.5 LRRK2 and Vesicle Trafficking

The study of LRRK2 localization benefits from specific antibodies. Apart from cytoskeletal interactions, LRRK2 is found in various neuronal membrane-bound

structures, including the Golgi apparatus, endoplasmic reticulum, lysosomes, mitochondria, plasma membrane, and partially in synaptic vesicles [128]. Multiple studies confirm LRRK2's presence in synaptic vesicles, suggesting its involvement in vesicular trafficking [129], [130]. Interactions between LRRK2 and Rab5b indicate a role in synaptic vesicle endocytosis regulation. LRRK2 dysregulation disrupts endocytosis, emphasizing the need for precise control. The identification of EndoA as a potential LRRK2 phospho-substrate supports this role [131], [132]. LRRK2 co-immunoprecipitates with presynaptic vesicular protein NSF. LRRK2 localization at synaptic vesicles regulates vesicle recycling and neurotransmitter release [129]. Silencing LRRK2 expression in primary cortical neurons increases the amplitude of post-synaptic EPSCs, suggesting enhanced vesicle recycling and mobility. However, in vivo studies show conflicting results regarding neurotransmitter release [129]. The removal of the native LRRK2 allele does not affect the basal levels of dopamine. Conversely, the increased expression of wild-type LRRK2 in transgenic mice results in elevated dopamine release in the striatum. Compromised dopamine release observed in various PD-associated mutant LRRK2 mouse models using diverse methodologies [133].

1.5.6 LRRK2's Involvement in Extrinsic and Inflammatory Signaling

LRRK2, part of the RIP kinase family alongside LRRK1, engages in multiple signaling pathways related to innate immunity, inflammation, and cell death. Mutant LRRK2-induced apoptosis in neurons involves caspase-8 and FADD, with PD-associated mutations enhancing their interaction and leading to caspase-8 activation in the brain [88]. The interaction between LRRK2 and FADD has been pinpointed to a specific motif within the LRRK2 N-terminal domain, underscoring its relevance in PD pathogenesis. Additionally, LRRK2 mutations in a non-coding region have been linked to elevated susceptibility to Crohn’s disease [134], highlighting its role in inflammatory signaling. LRRK2 deficiency exacerbates experimental colitis induced by dextran sodium sulfate (DSS), indicating its regulatory function in intestinal inflammation. Additionally, LRRK2 interacts with the NFAT, inhibiting its nuclear translocation and transcriptional activity. NFAT, known for its role in inflammatory responses and cytokine regulation, is implicated in inflammatory bowel disease, suggesting a potential link between LRRK2 and intestinal inflammation. Microglia expressing mutant LRRK2 demonstrate heightened levels of inflammatory cytokines, including TNF-α, and diminished anti-inflammatory cytokines, implicating LRRK2 in neuroinflammation. Pharmacological inhibition or knockdown of LRRK2 mitigates the lipopolysaccharide (LPS)-induced inflammatory response in microglia, underscoring its involvement in neuroinflammatory pathways

[135]. Moreover, IFN-γ treatment induces LRRK2 expression in peripheral immune cells [136].

1.5.7 LRRK2 and Mitochondrial Dysfunction

Mitochondrial dysfunction is a hallmark of PD, evident from various studies including the forfeiture of mitochondrial membrane [137] probable in cells from PD patients and the use of mitochondrial toxins to model the disease [138]. Moreover, emerging evidence suggests that LRRK2, a protein implicated in PD, modulates mitochondrial function. Studies have demonstrated the localization of a portion of cellular LRRK2 to mitochondria, with observed mitochondrial dysfunction in clinical tissues and PD models associated with LRRK2 mutation [128], [139]. In fibroblasts from PD patients resounding the G2019S LRRK2 mutation, mitochondrial ATP production and total cellular ATP levels are reduced compared to controls. Morphological analysis revealed alterations suggestive of impaired mitochondrial fission. Further investigations confirmed decreased ATP levels, reduced mitochondrial membrane potential, and increased oxygen consumption in patient cells. Interestingly, deficits in mitochondrial function in cells expressing LRRK2 mutations can be rescued by inhibiting LRRK2 kinase activity. Nevertheless, discrepancies exist regarding the mechanism underlying LRRK2-mediated mitochondrial dysfunction. Some investigations propose a direct physical interaction between LRRK2 and proteins involved in mitochondrial fission. proteins, leading to increased mitochondrial fragmentation, while others propose an interaction between LRRK2 and proteins regulating mitochondrial membrane permeability [140], [141]. Additionally, LRRK2 has been demonstrated to interact with proteins implicated in the assembly of the mitochondrial permeability transition pore, consequently impeding its maturation and translocation within mitochondria. Furthermore, dopamine LRRK2 mutant iPSC-derived neurons exhibit perturbed characteristics mitochondrial function and increased vulnerability to mitochondrial stressors. Interestingly, treatment with an LRRK2 kinase inhibitor protects against the loss of dopamine neurons induced by mitochondrial stressors, indicating a potential therapeutic strategy [142].

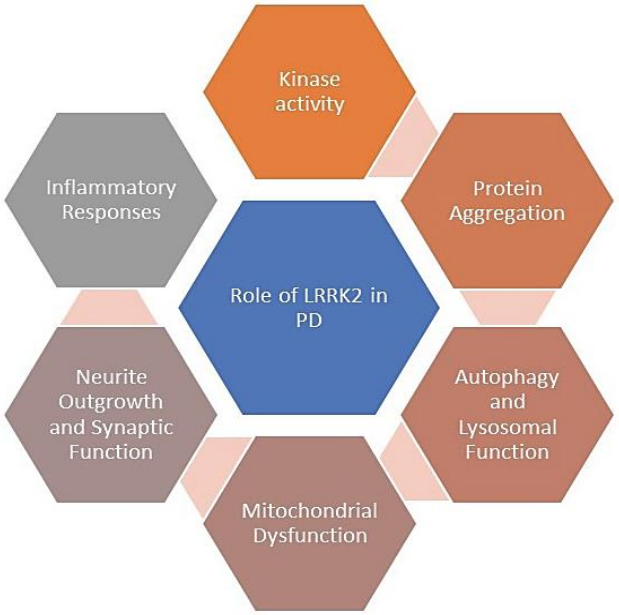

Fig 1.3: Different types of roles of LRRK2 in PD

1.6 Phytochemicals as a Potent Therapeutics for PD Management

A range of pharmaceutical interventions, including L-dopa, COMT inhibitors, MAO-B inhibitors, and dopamine agonists, are presently employed in the management of PD. Nonetheless, these pharmacotherapies primarily address dopamine deficiency and frequently fail to fully ameliorate symptoms or arrest disease progression [143], [144]. Consequently, they often fall short of meeting patients' long-term therapeutic expectations. This inherent limitation has sparked scientific inquiry into natural compounds, renowned for their potent antioxidant and anti-inflammatory properties, often accompanied by minimal adverse effects [145], [146]. Phytochemicals, in particular, combat PD through multiple mechanisms: they suppress apoptosis (by reducing caspase-3, -8, and -9, and Bax/Bcl-2 levels), hinder synuclein deposition, mitigate dopaminergic neuron loss, and diminish the expression of proinflammatory cytokines (such as interleukin-1β, nuclear factor-κB, prostaglandin E2, and interleukin-6). Additionally, they address dopamine depletion and cellular inflammatory signaling, while enhancing antioxidant status and neurotrophic factors [145], [147], [148]. Medicinal plants, enriched with additional nutritional elements, not only offer health benefits but also augment nutritional value due to their impact on metabolic processes.

In the last decade, diverse bioactive constituents derived from medicinal plants have emerged as promising resources for PD drug research. Studies conducted between 2019 and 2023 have demonstrated the efficacy of certain notable medicinal plants and herbal formulations in managing PD. Previous research on these plants, predating 2019, has been extensively reviewed by other scholars. Moreover, polyphenols, terpenoids, and alkaloids have demonstrated potential benefits in in vitro and in vivo models of neurodegenerative disorders, including PD. Medicinal plants harbor numerous phytochemicals comprising a variety of secondary metabolites such as polyphenols (phenolic acids, anthocyanins, proanthocyanidins, flavanols, tannins), isoprenoids (sesquiterpenes, diterpenes, triterpenes, steroids, saponins), alkaloids (indole alkaloids, lysergic acid diethylamide, tropane alkaloids, ergot group), and fatty acids. These dynamic constituents interact with various enzymes and cell receptors. Although many medicinal plants have been traditionally utilized across cultures for treating cognitive disorders, only a few have undergone extensive study to validate the pharmacological basis of their medicinal effects [149], [150], [151].

1.6.1 Effect of Phytochemicals on α-Synuclein Aggregation

Alpha-synuclein (α-syn), a presynaptic protein consisting of 140 residues, plays a pivotal role in synaptic vesicle trafficking and fusion, as well as in regulating dopamine release at presynaptic terminals [152], [153]. In a normal human brain, the physiological concentration of a-syn is approximately 1 μM, and in cerebrospinal fluid, it is around 70 pM [154]. Under physiological conditions, α-syn exists as an unfolded monomer but adopts an α-helical secondary structure upon binding to lipid vesicles. However, destabilization of this protein leads to its misfolding and aggregation within neurons, making α-syn a significant therapeutic target [155], [156]. Strategies aimed at inhibiting its aggregation, oligomerization, and fibrillation are pivotal for modifying the progression of the disease [157], [158]. Recent investigations have showcased the neuroprotective effects of plant extracts and phytochemicals by targeting distinct stages of α-syn oligomerization and fibrillation. Identification of specific plant extracts and phytochemicals capable of inhibiting α-syn aggregation holds promise for the development of novel drugs for PD. These extracts and compounds have demonstrated the ability to hinder aggregation or fibril formation of oligomers and redirect α-syn oligomers into non-toxic pathways or their unstructured forms, positioning them as potential drug candidates for PD and related synucleinopathies [159]. The process of α-syn oligomerization and fibrillation is intricately linked to the onset and progression of PD, dementia with Lewy bodies (DLB), and related synucleinopathies, rendering it a compelling target for disease-modification therapies [157], [158]. Targeting α-syn aggregation, oligomerization, fibrillation, and propagation to mitigate its toxicity is essential for slowing or halting disease progression. Monomeric α-syn, as an upstream

form during the aggregation process, represents a promising therapeutic target in the pathogenesis of PD. Compounds possessing the capacity to stabilize, facilitate clearance, induce degradation of targeting misfolded proteins or inhibit α-syn aggregates show clinical promise in Parkinson's therapeutics. [159]. Multiple botanical extracts have demonstrated neuroprotective properties in PD by intervening in experimental models display diverse α-synuclein conformations, from fibrillation to oligomerization, across pathological phases. [159]. Traditional medicine focuses on α-synuclein with plant extracts rich in phytochemicals might present benefits through dietary interventions, providing synergistic actions and enhanced therapeutic outcomes owing to their polypharmacological attributes [161]. The reduction of α-syn toxicity by botanical extracts corroborates the traditional assertions of their medicinal advantages and advocates for the incorporation of these plants into diets for neuroprotective purposes. Phytochemicals, functioning as non-nutritive secondary metabolites, are widely employed in the process of drug discovery and development due to their expansive structural variability, which offers lead structures for novel pharmaceuticals.. These compounds belong to various classes, including alkaloids, saponins, carotenoids, lignans, and glycosides. Dietary intake of polyphenolic compounds has been demonstrated to confer protection against neurodegeneration, as evidenced by numerous epidemiological and experimental studies. These polyphenols inhibit α-syn aggregation and fibrillation as well as the formation of amyloid protofilaments and fibrils, thereby providing protective effects in neurodegenerative diseases [159].

1.6.2 Effect of Phytochemical in Gut Microbiota on α-Synuclein Aggregation

Targeting the aggregation, oligomerization, fibrillation, and propagation of α-syn to mitigate its toxicity represents a crucial therapeutic goal for attenuating or arresting disease advancement [157], [158]. Recent studies have underscored the potential neuroprotective properties of plant extracts and phytochemicals in PD owing to their antioxidative and anti-inflammatory characteristics [159]. Within dopaminergic neurons, the formation of intracytoplasmic inclusions containing α-syn, synphilin-1, and ubiquitin initiates LB formation, a PD hallmark. α-synuclein aggregation begins with dimerization, progressing to oligomers, protofibrils, and β-sheet-enriched fibrils, eventually forming LB constituents, the end-stage fibrils, and aggregated α-syn [157], [158]. Consequently, the oligomerization of α-syn monomers serves as the pivotal initial step in the multistep process of α-syn-induced neuronal toxicity, leading to the genesis of intracytoplasmic inclusions and fibrils. Various plant extracts have been identified to impede the oligomerization and fibrillization of α-syn, positioning them as vital therapeutic candidates in PD. These extracts, showing

neuroprotective effects in PD, target various pathological stages of α-syn, from fibrillation to oligomerization [159].

The LRRK2 G2019S mutation significantly affects the lysosomal degradation of α-syn by reducing LAMP2A (lysosome-associated membrane protein type 2A) levels, thereby impairing chaperone-mediated autophagy (CMA), a selective lysosomal degradation pathway. However, α-syn accumulation is not always due to gene mutations; aggregates can form before disease onset through other means [162]. Research suggests a correlation between the GI tract and the CNS, denoted The gut-brain axis: a bidirectional communication pathway via the vagus nerve (the tenth cranial nerve). Dysbiosis, a change in the gut's microbial population, can lead to the production of certain metabolites that cause α-syn buildup in the gut, which can then be transported to the brain via the vagus nerve. Within the brain, α-syn can deplete dopamine neurons, altering signaling and causing disease [163], [164].

Research indicates that the gut microbiota influences both CNS and ENSss functions, underscoring the significant role of metabolites and cellular components originating from the gut in maintaining brain balance [164]. Metabolites and neurotransmitters produced by gut microbiota communicate with the CNS/ENS, affecting neuromodulation and regulating critical processes like neurogenesis, blood-brain barrier integrity, myelination, synaptic pruning, and glial cell function [165]. Studies suggest that misfolded proteins associated with neurodegenerative diseases can travel from the gut to the brain. In PD, for instance, misfolded α-syn aggregates have been detected in the ENS. Dysbiosis exacerbates this process by facilitating the buildup and transfer of misfolded proteins, highlighting the complex connection between gut health, protein aggregation, and neurodegeneration [164], [166].

Phytochemicals, under their antioxidant, anti-inflammatory, antiapoptotic, and neuroprotective properties, confer therapeutic advantages [167]. Compounds targeting misfolded proteins and α-syn aggregates offer promising therapeutic avenues for PD.. Targeting the aggregation, oligomerization, and fibrillation of α-syn is a crucial strategy for modifying disease progression in PD [153], [154]. Plant extracts inhibit α-syn oligomerization and fibrillation, showing neuroprotective effects. These natural compounds target different stages of α-syn formation, offering selective molecules for developing new PD treatments [155]. Plant extracts and phytochemicals have exhibited the capacity to hinder the aggregation or fibrillation process of α-syn oligomers. Additionally, they appear

to redirect α-syn oligomerization into an amorphous state or facilitate non-toxic pathways, rendering them auspicious candidates for pharmacological intervention in PD and associated synucleinopathies [159]. Cinnamon extract inhibits α-syn aggregation, stabilizes oligomers in A53T PD [161]. This novel approach targets α-syn aggregates in the gut, suggesting that these phytochemicals, absorbed through the gastrointestinal tract, could combat the disease without the need for blood-brain barrier-permeable drugs [166].

CHAPTER 2

METHODOLOGY

2.1 Source

The database used: PubMed, IMPPAT 2.0, PubChem, UniProt, Protein Data Bank (PDB)

Software used: PyRx, Biovia Discovery Studio Visualizer, Pymol, SwissADME, OpenBabel, CarcinoPred-EL, PkCSM, CB-Dock2

2.2 Workflow

2.2.1 Phytochemicals Showing BBB Permeability

A literature survey identified LRRK2 G2019S as a potential target for repurposing an antagonist to specifically address the abnormal indirect signaling associated with LRRK2 G2019S in Parkinson's disease (PD). To identify suitable candidates, a list of phytochemicals known for their Anti-Parkinson, Anti-Cancer, Anti-Inflammatory and other therapeutic properties was compiled from the literature. Their chemical structures were retrieved from IMPPAT 2.0. Among these, the phytochemicals with the ability to penetrate the BBB were selected as ligands for further investigation. The protocol flowchart is illustrated in Figure 3.1.

2.2.2 Phytochemicals Targeting α-Synuclein Aggregation

A literature survey identified LRRK2 G2019S as a potential target molecule for repurposing an antagonist to specifically address the abnormal indirect signaling related to α-synuclein aggregation, which is a major cause of PD. To identify suitable candidates, a list of phytochemicals known to target alpha-synuclein aggregation was compiled through a literature review, and their chemical structures were retrieved from IMPPAT 2.0. Among these phytochemicals, those with high GI absorption were filtered out as ligands for further investigation in the study. The protocol flowchart is illustrated in Figure 3.2.

IMMPAT2.0

IMPPAT 2.0 (Indian Medicinal Plants, Phytochemistry And Therapeutics 2.0) is the most extensive manually curated database of phytochemicals, created by digitizing vast amounts of data on traditional Indian medicinal plants. This platform emphasizes the relationships between plants, their parts, phytochemicals, and therapeutic uses. As an integrated resource, IMPPAT 2.0 showcases the knowledge embedded in traditional Indian medicine and aids in the discovery of natural product-based drugs.

PubChem

PubChem, managed by the NCBI, which operates under the United States National Institutes of Health (NIH), is a comprehensive repository of chemical compounds and their biological activities. It provides free access via a web interface, allowing users to download compound structures and descriptive datasets through FTP. PubChem contains diverse substance descriptions and small molecules, typically with fewer than 100 atoms and 1,000 bonds. Its database continually expands with contributions from over 80 database vendors.

2.3 Data Extraction

From the RCSB PDB official website, the 3-D structure of LRRK2 G2019S (PDB ID: 7LI3) was obtained (https://www.rcsb.org/). A literature survey identified 14 medicinal plants with wide choice of therapeutic properties such as anticancer, neuroprotective, anti-inflammatory, antimalarial, anti-allergic, anti-diabetic, anti-nociceptive, analgesic, anti-microbial, cytotoxic, antioxidant, antilipidimic, hepatoprotective, vasorelaxant, anti-tumor, anti-bacterial, anti-proliferative, anti-fungal, antiulcer, antidiarrhoeal, immunomodulatory, antipyretic, anti-plasmodic, antihistaminic, anti-helminthic, astringent, anti-hyperglycaemic, antispasmodic and others mentioned in the below table and phytochemical compounds targeting α-synuclein aggregation. The 3D structures of these phytochemical compounds specific for each plant were retrieved from IMPAAT 2.0 by making individual entry. Additionally, the structure of a well-known LRRK2 G2019S inhibitor, Serotobenine, (IMPHY002029) was extracted from the IMPPAT 2.0 in 3D .sdf format. The ChEMBL database was utilized to evaluate various properties of the ligands, such as molecular weight. For FDA-approved drugs, the compounds were downloaded from DrugBank, using venetoclax as the reference drug with its structure obtained from PubChem. The target protein LRRK2 G2019S is downloaded from Protein Data Bank (PDB) in .pdb format.

Table 2.1: List of Medicinal plants with their therapeutic properties

S.No.	Name of medicinal plant	Family	Number of phytochemical entries	Source of phytochemical	Activity	Reference
1.	Albizia lebbeck	Fabaceae	108	Bark, flower, fruit, leaf, root, seed, wood	Anticancer, anti-nociceptive, anti-inflammatory, antimalarial, antiallergic, neuroprotective	[168], [169], [170]
2.	Asparagus officinalis	Asparagaceae	51	flower, leaf, root, seed, shoot	Anti-diabetic, anti-cancer, anti-fungal, antimicrobial	[171], [172], [173]
3.	Asparagus racemosus	Liliaceae	40	Bark, flower, fruit, leaf, root, wood,	Antiulcer, antioxidant, and antidiarrhoeal, antidiabetic and immunomodulatory, antitumor	[174], [175]
4.	Bauhinia racemosa	Fabaceae	20	Bark, root, seed, stem, wood	Analgesic, antipyretic, anti–inflammatory, anti-plasmodic, antimicrobial, antihistaminic	[176], [177], [178]
5.	Butea monosperma	Fabaceae	77	Bark, flower, plant exudate, root, seed, whole plant	Anti-tumor, anti-microbial, anti-helmintic, anti-inflammatory, astringent	[179], [180]
6.	Cedrus deodara	Pinaceae	189	Bark, flower, leaf, plant exudate, root, seed, wood, whole plant	Anti-inflammatory, analgesic, , antibacterial, insecticidal, anti-apoptotic, immunomodulatory, anticonvulsant, anti-cancer	[181]
7.	Croton tiglium	Euphorbiaceae	33	Seed	Anti-bacterial, anti-fungal, analgesic, anti-inflammatory, anti-HIV, anti-tumor	[182], [183], [184]
8.	Datura metel	Solanaceae	104	Aerial, part, bark, flower, fruit, leaf, root, seed, stem, whole plant	Anti-proliferative, anti-inflammatory, antioxidant, antipyretic, and analgesic	[185], [186], [187]
9.	Euphorbia hirta	Euphorbiaceae	129	Aerial, part, bark, flower, leaf, plant exudate, root, stem, whole	Immunomodulatory, anti-inflammatory, analgesic, anti-tumor	[188]

10.	Moringa oliefera	Moringaceae	200	Bark, flower, fruit, leaf, root, seed, stem, whole plant	Antioxidant, anti-cancer, anti-inflammatory	[189]
11.	Plantago major	Plantaginaceae	46	Aerial part, flower, leaf, root, seed, whole plant	Hepatoprotective, Anti-hypercholesteremia, Anti-atherosclerosis, anti-inflammatory, analgesic, antifungal, antiviral, anti-bacterial, anti-cancer	[190], [191], [192]
12.	Taxus wallichiana	Taxaceae	181	Bark, fruit, leaf, Root, stem, wood	Analgesic, anti-inflammatory, immunomodulatory, antispasmodic antiallergic, anticonvulsant, antiosteoporotic, anticociceptive, antimicrobial, antiplatelet, antipyretic	[193], [194], [195]
13.	Urtica dioica	Urticaceae	69	Flower, leaf, plant cells/culture, rhizome, root, trichome	Antioxidant, Anti-Inflammatory, Hypoglycemic, Antiulcer, Cardiovascular protective, Repression of prostate-cell metabolism and proliferation	[196], [197]
14.	Vitex negundo	Verbenaceae	228	Bark, flower, fruit, leaf, root, seed, stem	Antihelmintic, anti-inflammatory, anti-proliferative, antioxidant	[198]

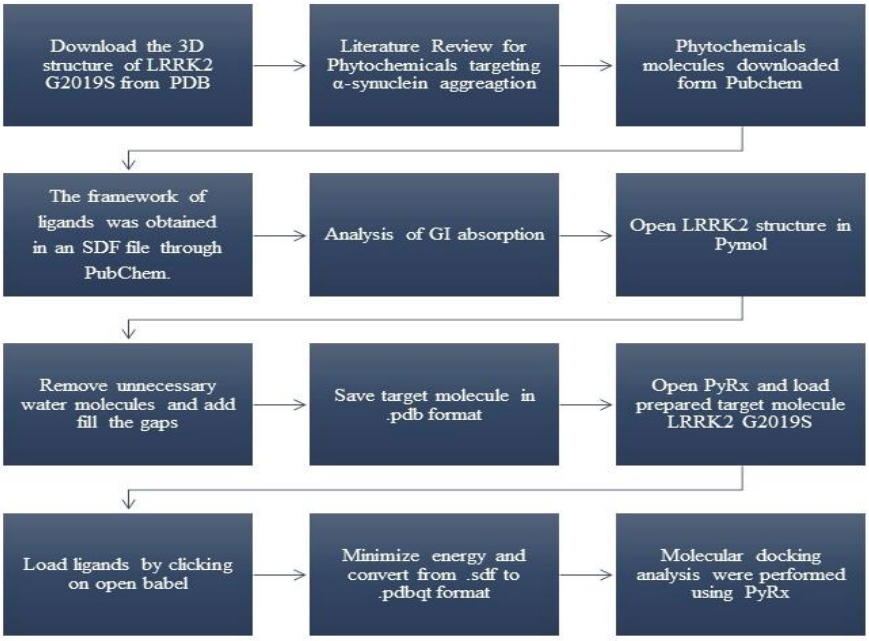

Fig 2.1: Flowchart of steps involved in molecular docking of Phytochemicals targeting α-synuclein aggregation

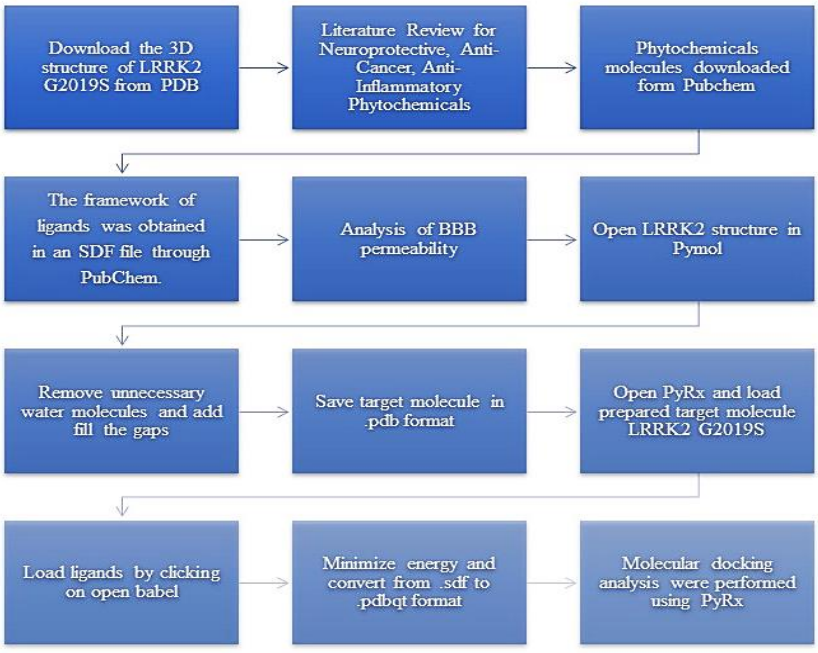

Fig 2.2: Flowchart of steps involved in molecular docking of Phytochemicals from Medicinal plants

2.4 BBB Permeability Analysis

Out of the 1580 phytochemicals, only 390 were found to cross the BBB. This BBB permeability analysis was conducted using an online tool specifically designed for predicting BBB permeability Swiss ADME. The downloaded compounds were converted from .pdb files to SMILES format using Open Babel, a versatile and open-source toolbox. Subsequently, all these compounds were uploaded to Swiss ADME to assess their BBB permeability. For the remaining compounds that were BBB negative, and some 105 phytochemical compounds GI absorption was considered as a factor for targeting alpha-synuclein aggregation. For FDA-approved drugs, out of a pool of 3,674 pharmaceutical compounds subjected to testing, only 16 exhibited permeability across the blood BBB.

2.5 Molecular Docking

Afterwards, the interaction between the receptor and ligand was scrutinized through molecular docking using the PyRx web server. Subsequent procedures were then conducted.

2.5.1 Preparation of the Target Receptors

Preparation of the target receptors involved processing the LRRK2 protein cohort using the Discovery Studio 2024 client. The three dimensional structure of LRRK2 G2019S having PDB ID: 7LI3, resolution of 3.80 Å in its Hydrolase Transferase configuration was retrieved from PDB in .sdf format. After eliminating unnecessary ligands like removing bound complex molecules, non–polar hydrogens, all heteroatoms and non-essential water molecules, the sophisticated protein structure was saved as a .pdb file. Using PyMOL Win, redundant water molecules were removed from LRRK2, and any gaps in chains A and B were filled with a UniProt sequence. Polar hydrogens and gasteiger charges were added. The resulting modified protein was stored as a .pdb file. Subsequently, PyRx was employed to import the LRRK2 .pdb file and transform it into a macromolecule.

2.5.2 Preparation of the Ligand Molecules

In the quest to find a promising inhibitory drug for LRRK2, a collection of 1580 phytochemical ligands was utilized for docking experiments. Using Open Babel within PyRx, these ligands were transformed from .sdf to PDBQT format. Following energy minimization, of the ligands that were converted from .sdf to .pdbqt format using Open Babel within PyRx. In case of FDA approved, once the

structure of drugs were obtained, Open Babel was used to convert the retrieved compounds into .sdf format for docking.

2.5.3 Molecular Docking Studies

PyRx: PyRx, an open-source software, is utilized for the virtual screening of libraries to identify potential drug targets. It comprises a substantial collection of various software tools, making it an invaluable asset for computer-aided drug discovery. In the study conducted, Open Babel was used for importing ligand files, and Autodock Vina was employed for docking purposes.

In this study Docking is performed after the preparation of ligands and protein. Open Babel is employed within PyRx for importing ligands and converts the ligands to .pdbqt format after energy minimization, while AutoDock Vina is used for docking simulations. After preparation, both the protein and ligand will be visible in the AutoDock Tab. The process begins with loading the protein molecule and converting it into a macromolecule. Following this, the ligands and protein are selected, and the grid box dimensions are adjusted using the forward option to ensure the entire protein is encompassed within the grid box. Molecular docking experiments were carried out using the AutoDock Vina program integrated into the PyRx platform. Blind docking procedures were performed using the Vina methods incorporated within PyRx. Once the docking is completed, the results are saved as output files and .csv files for further analysis.

2.5.4 Docking Analysis

To select efficient ligands, .csv files for each plant were analyzed, and potential ligands were identified based on their docking scores. To identify efficient FDA-approved drugs, the drugs with highest affinity were first selected by analyzing .csv files. Among these, the drugs that are permeable to the blood-brain barrier (BBB) were then identified and subjected to further analysis.

2.5.5 Docking Results Validation

To validate the results, CB-Dock2, an advanced blind docking server designed for virtual screening, was utilized. The results for phytocompounds and FDA-approved drugs are presented in the Table 3.5 & 3.6.

2.5.6 Analyzing Protein-Ligand Interactions

The output files of the selected ligands were further analyzed. The interactions were scrutinized using Discovery Studio Visualizer and PyMOL for 2D and 3D with the protein. Compounds showing a Root-Mean-Square-Deviation (RMSD) below 1 Å and binding free energy exceeding -9.00 kcal/mol were prioritized and assessed for BBB permeability using an online predictor cbligand.org. Out of 1580 initially tested phytochemical compounds, only 390 demonstrated BBB permeability. However, only 10 phytocompounds with binding energy $\leq$ 9.00 kcal/mol were preferred. Additionally, out of 105 alpha-synuclein targeting molecules, only 12 were selected. These platforms facilitate the visualization of ligand binding sites on proteins, providing detailed information on the types and quantity of interactions, and identifying the specific amino acids engaged in these interactions.

2.5.7 SwissADME-based ADME Assessments for the Ligand

SwissADME was employed for the prediction of ADMET properties, whereas CarcinoPred-EL and PkCSM tools were utilized to predict carcinogenicity and toxicity, respectively. ADME analysis was performed on a group of six selected physicochemical compounds using the SWISS-ADME framework. This computational tool facilitated an in-depth investigation into the pharmacokinetic properties, elucidating the processes of absorption, distribution, metabolism, and excretion of these compounds. The study scrutinized critical constraints comprising pharmacokinetics, GI absorption probability, BBB permeability, P-gp substrate status, compliance with Lipinski's Rule, any infractions thereof, aqueous solubility, lipophilicity, and bioavailability. Lipinski's Rule of 5 consists of parameters implemented to assess the drug-likeness of a molecule. For oral bioavailability, a compound is considered favourable if it meets specific criteria: molecular mass below 500 Daltons, $\leq$ 5 hydrogen bond-donating sites, $\leq$ 10 hydrogen bond-accepting entities, and a calculated logarithm of the partition coefficient (LogP) not exceeding five. These criteria act as benchmarks for evaluating the likelihood of a compound possessing optimal pharmacokinetic attributes for effective oral delivery.

2.5.8 Pharmacokinetics Predictions

Pharmacokinetic and drug-likeness parameters are assayed of the lead compounds, characterized by interaction scores greater than -9.00 kcal/mol, were evaluated by assessing their ADME predictions using SwissADME [199]. Additionally, CarcinoPred-EL [200] a web-based tool employing ensemble

learning methods predicted the carcinogenic potential of compounds. The oral rat acute toxicity LD50 was determined using PkCSM, a graph-based method for the toxicity calculation tool, that also predicts the maximum tolerated dose in humans [201].

CHAPTER 3

RESULT & DISCUSSION

3.1 Docking Results

The blood-brain permeable phytochemicals derived from 14 Indian medicinal plants were subjected to molecular docking along with the reference compound Serotobenine. The binding energy of 8.8 kcal/mol was obtained for the reference compound with LRRK2 G2019S. For identification of an effective LRRK2 G2019S inhibitor the compounds with a binding affinity of 9.0 kcal/mol or lower were considered. Among the phytochemicals sourced from 14 different plants, only those from 4 plants met the desired criteria outlined in the Table. The phytochemical with the most negative docking score, indicating the most stable ligand-protein complex, was derived from the Asparagus officinalis plant with an IMPPAT ID, IMPHY003711. This compound emerged as the most potent inhibitor, boasting a docking score of -10.5 kcal/mol and an RMSD value of 0.0 Å. For validation and further analysis, the top five compounds with the highest binding scores were selected. For FDA-approved drugs, Ponatinib showed the most negative binding energy score of -10 kcal/mol, while the reference compound scored -8.0 kcal/mol. A threshold of > -9.0 kcal/mol was set based on the number of drugs with high affinity. Compounds within this range were then analyzed for BBB permeability using Swiss ADME. Five compounds met the criteria, with the top three drugs Ponatinib, Mosapramine and Drospirenone with binding affinity -10.0 kcal/mol, -9.8 kcal/mol and -9.7 kcal/mol were selected for further investigation.

Table 3.1: Phytochemicals obtained from Medicinal Plants with high docking score

Compound	Docking score	Solubility Class (SILICOS-IT)	GI absorption	BBB permeant	P-gp substrate	IMPPAT Phytochemical identifier	Medicinal Plant
Isowithametelin	-10.3	Moderately soluble	High	Yes	Yes	IMPHY010687	Datura metel
Datumetelin	-9.1	Moderately soluble	High	Yes	Yes	IMPHY004278	Datura metel
Daturilinol	-9.1	Moderately soluble	High	Yes	Yes	IMPHY008964	Datura metel

Sarsasapogenin	-9.2	Moderately soluble	High	Yes	No	IMPHY012274	Asparagus officinalis
Yamogenin	-10.5	Moderately soluble	High	Yes	No	IMPHY003711	Asparagus officinalis
Diosgenin	-9.9	Moderately soluble	High	Yes	No	IMPHY003681	Asparagus racemosus
Withametelin B	10.1	Moderately soluble	High	Yes	Yes	IMPHY009120	Datura metel
Daturametelin D	-9.9	Moderately soluble	High	Yes	Yes	IMPHY004277	Datura metel
Withametelin	-10.3	Moderately soluble	High	Yes	Yes	IMPHY003277	Datura metel
Luteoxanthin	-9.5	Moderately soluble	High	Yes	Yes	IMPHY002029	Utrica diocia

Table 3.2: Phytochemicals compounds targeting α-synuclein aggregation with high docking score

Compound	**Docking score**	**Solubility class**	**GI absorption**	**BBB permeant**	**P-gp substrate**
Withanolide A	-10.2	Moderately soluble	High	No	Yes
Hederagenin	-9.8	Moderately soluble	High	No	Yes
3-O-Demethylswertipunicoside	-10.5	Moderately soluble	Low	No	No
Hinokiflavone	-10.4	Moderately soluble	Low	No	No
Astaxanthin	-10.3	Moderately soluble	Low	No	Yes
Swertipunicoside	-10.1	Moderately soluble	Low	No	No
Hypericin	-9.5	Poorly soluble	Low	No	No
Rutin	-9.1	Soluble	Low	No	Yes
1,8-Bis((2R,3R)-3,5,7-trihydroxy-2H-1-benzopyran-2-yl)-3,4,6-trihydroxy-5H-benzocyclohepten-5-one	-9.5	Moderately soluble	Low	No	No
Beta-Amyrin	-9.4	Poorly soluble	Low	No	No
Icariin	-9.2	Moderately soluble	Low	No	Yes
Celastrol	-9.1	Moderately soluble	Low	No	Yes

Table 3.3: Phytochemical hit compounds: drug likeliness and pharmacokinetics

IMPAAT ID	GI absorption	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
IMPHY002029	High	No	No	No	No	No
IMPHY010687	High	No	No	Yes	No	No
IMPHY003277	High	No	No	Yes	No	No
IMPHY009120	High	No	No	Yes	No	No
IMPHY003681	High	No	No	No	No	No
IMPHY008964	High	No	No	Yes	No	No
IMPHY012274	High	No	No	No	No	No
IMPHY004278	High	No	No	No	No	No
IMPHY003711	High	No	No	No	No	No
IMPHY004277	High	No	No	No	No	No

Table 3.4: Hit phytochemicals targeting α-synuclein aggregation: drug likeliness and pharmacokinetics

Compound	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	GI absorption
Withanolide A	No	No	No	No	No	High
Hederagenin	No	No	No	No	No	High
3-O-Demethylswertipunicoside	No	No	Yes	No	No	Low
Hinokiflavone	No	No	Yes	No	No	Low
Astaxanthin	No	No	No	No	No	Low
Swertipunicoside	No	No	Yes	No	No	Low
Hypericin	No	Yes	Yes	No	No	Low
Rutin	No	No	No	No	No	Low
1,8-Bis((2R,3R)-3,5,7-trihydroxy-2H-1-benzopyran-2-yl)-3,4,6-trihydroxy-5H-benzocyclohepten-5-one	No	No	Yes	No	Yes	Low
Beta-Amyrin	No	No	No	No	No	Low
Icariin	No	No	No	No	No	Low
Celastrol	No	No	Yes	No	Yes	Low

1. Validation results

Upon validating the top five phytochemicals obtained from Indian Medicinal plants and the top three FDA-approved drug candidates with CB-Dock2, notably significant values were obtained, with differences between the scores from PyRx and CB-Dock2 being approximately ≤ 0.5 kcal/mol and the docking score of -7.3 kcal/mol for curcumin and -8.0 for venetoclax, the reference compound. The docking scores of these compounds are mentioned in the below Table.

Table 3.5: Top five phytochemicals scored in PyRx, validated by CB Dock 2

Target Protein	IMPPAT ID	PyRx	CB Dock 2
LRRK2	IMPHY003711	-10.5	-10.0
	IMPHY010687	-10.3	-10.4
	IMPHY003277	-10.3	-9.8
	IMPHY009120	-10.1	-9.4
	IMPHY003681	-9.9	-10.0
	Reference compound	-8.8	-8.5

Table 3.6: Top three FDA-approved drugs scored in PyRx, validated by CB Dock 2

Target Protein	Compound	PyRx	CB Dock 2
LRRK2	Ponatinib	-10.0	-9.2
	Mosapramine	-9.8	-9.9
	Drospirenone	-9.7	-8.6
	Reference compound	-8.8	-8.0

2. Results of Protein-Ligand Interaction Analysis

The interactions for the top five compounds, along with a reference compound, were analyzed using Discovery Studio and PyMOL. These analyses utilized output files generated by PyRx. Additionally, interaction diagrams obtained are also included.

3. ADME Analysis Results

The top compounds were evaluated for ADME using the Swiss ADME software. The resulting data, which includes the physicochemical properties, drug-likeness, and pharmacokinetics of the identified compounds, are presented in the table. The BIOLED-EGG images and the bioavailability radar diagrams of these phytochemicals were also recorded and referenced in the figure.

Table 3.7: Physicochemical properties of hit Phytochemicals compounds

Properties	Drug name				
	Isowithametelin	**Yamogenin**	**Withametelin**	**Diosgenin**	**Withametelin B**
Molecular weight (g/mol)	436.58	414.62	436.58	414.63	452.59
Hydrogen-bond donors	0	1	0	1	1
Hydrogen-bond acceptors	4	3	4	3	5
Molar refractivity	124.69	121.59	124.69	119.40	125.85
Topological polar surface area (Å2)	52.60	38.69	52.60	38.69	72.83
Lipinski's rule of five	Yes; 1 violation	Yes; 1 violation	Yes; 1 violation	Yes; 1 violation	Yes; 0 violations
Bioavailability score	0.55	0.55	0.55	0.55	0.55
Log P [SILICOS-IT]	4.91	4.29	4.91	5.71	4.01
Solubility	-5.09	-4.49	-5.09	-5.38	-4.27

Table 3.8: Physicochemical properties of hit Phytochemicals compounds targeting α-synuclein

Properties	Drug name	
	Withanolide A	**Hederagenin**
Molecular weight (g/mol)	470.60	472.70
Hydrogen-bond donors	2	3
Hydrogen-bond acceptors	6	4
Molar refractivity	127.53	137.82
Topological polar surface area (Å2)	96.36	77.76
Lipinski's rule of five	Yes; 0 violation	Yes; 1 violation
Bioavailability score	0.55	0.56
Log P [SILICOS-IT]	3.78	5.24
Solubility	-3.78	-5.55

Table 3.9: Physicochemical & Pharmacological properties of hit FDA-approved drugs

Compound	**Molecular weight (g/mol)**	**Hydrogen-bond donors**	**Hydrogen-bond acceptors**	**Topological polar surface area (Å2)**	**Solubility**	**Log P [SILICOS-IT]**
Ponatinib	565.32	1	8	65.77	-8.46	4.49
Mosapramine	479.06	1	3	38.82	-7.90	4.23
Drospirenone	366.49	0	3	43.37	-4.31	4.12

Compound	Lipinski's rule of five	Bioavailability score	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
Ponatinib	Yes; 1 violation	0.55	No	Yes	Yes	Yes	No
Mosapramine	Yes; 1 violation	0.55	No	No	No	Yes	Yes
Drospirenone	Yes; 1 violation	0.55	No	No	No	No	No

4. Toxicity and Carcinogenicity analysis

The summary of results for selected phytochemical compounds were presented in Table.

Table 3.10: Toxicity of hit Phytochemicals compounds

Phytochemicals with high BBB permeability				
Compound	**Ames toxicity**	**Oral rat acute toxicity LD50 (mol/kg)**	**Max. tolerated dose human (log mg/kg/day)**	**Carcinogenicity**
Isowithametelin	No	1.926	-0.446	No
Datumetelin	No	2.099	-0.261	No
Daturilinol	No	2.024	-0.453	No
Sarsasapogenin	No	2.041	-0.388	No
Yamogenin	No	1.855	-0.461	No
Diosgenin	No	1.855	-0.461	No
Withametelin B	No	2.193	-0.737	No
Daturametelin D	No	2.099	-0.261	No
Withametelin	No	1.926	-0.446	No
Luteoxanthin	No	2.192	-0.847	No
Phytochemicals Targeting α-synuclein aggregation				
Withanolide A	No	2.987	-0.589	No
Hederagenin	No	2.856	0.139	No

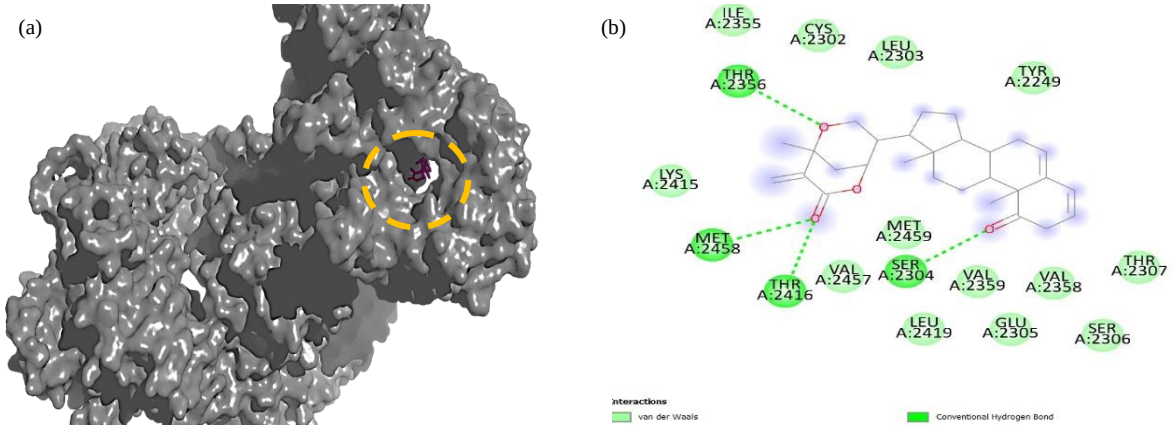

Fig 3.1 (a) The three-dimensional representation depicting the binding mode of Isowithametelin with LRRK2 G2019S. (b) The two-dimensional illustration showcasing the binding pattern of the proposed phytochemical, Isowithametelin, with the LRRK2 G2019S protein.

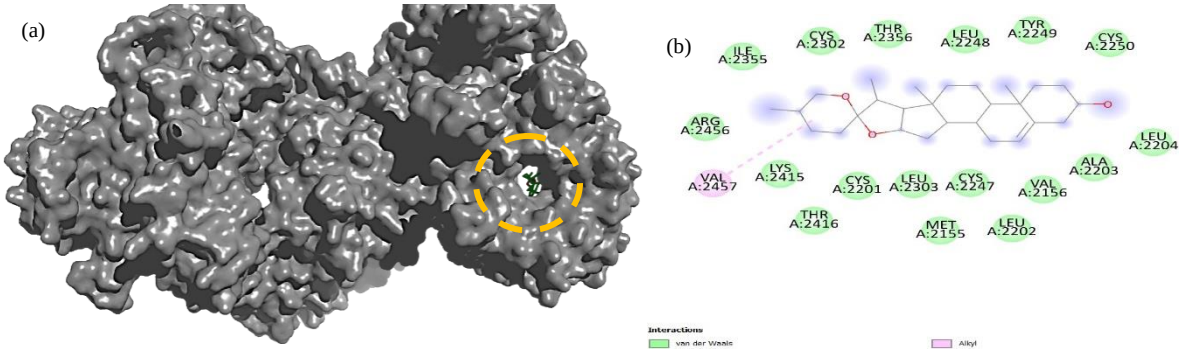

Fig 3.2 (a) The three-dimensional representation depicting the binding mode of Yamogenin with LRRK2 G2019S. (b) The two-dimensional illustration showcasing the binding pattern of the proposed phytochemical, Yamogenin, with the LRRK2 G2019S protein.

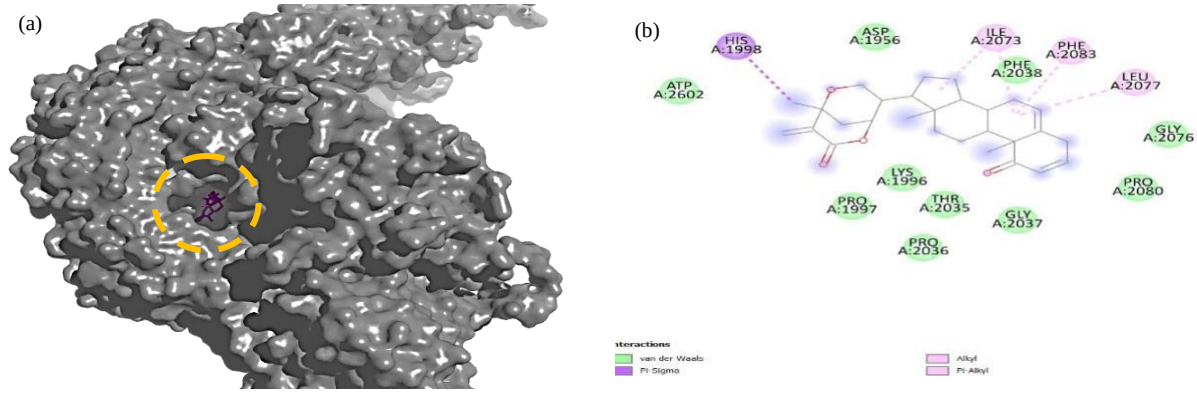

Fig 3.3 (a) The three-dimensional representation depicting the binding mode of Withametelin with LRRK2 G2019S. (b) The two-dimensional illustration showcasing the binding pattern of the proposed phytochemical, Withametelin, with the LRRK2 G2019S protein.

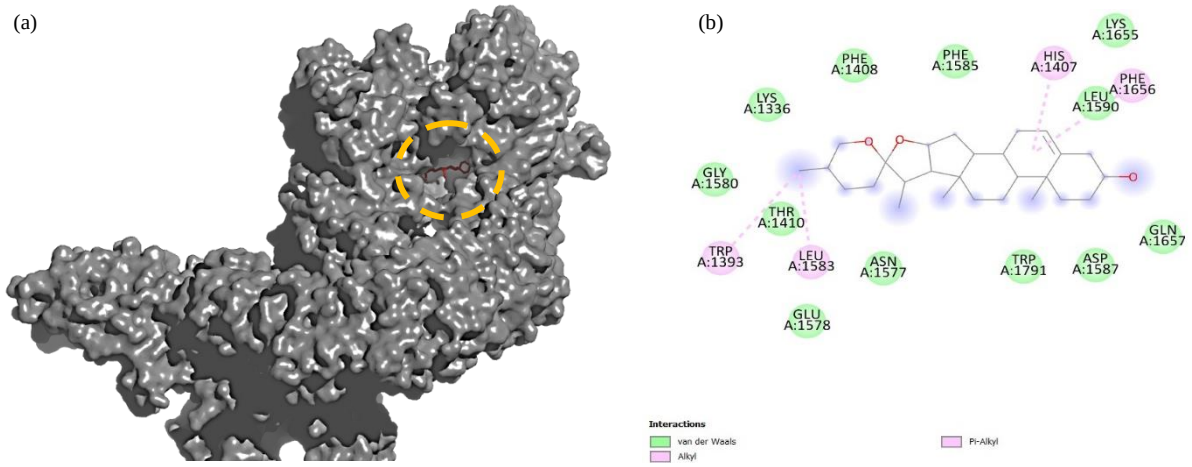

Fig 3.4 (a) The three-dimensional representation depicting the binding mode of Diosgenin with LRRK2 G2019S. (b) The two-dimensional illustration showcasing the binding pattern of the proposed phytochemical, Diosgenin, with the LRRK2 G2019S protein.

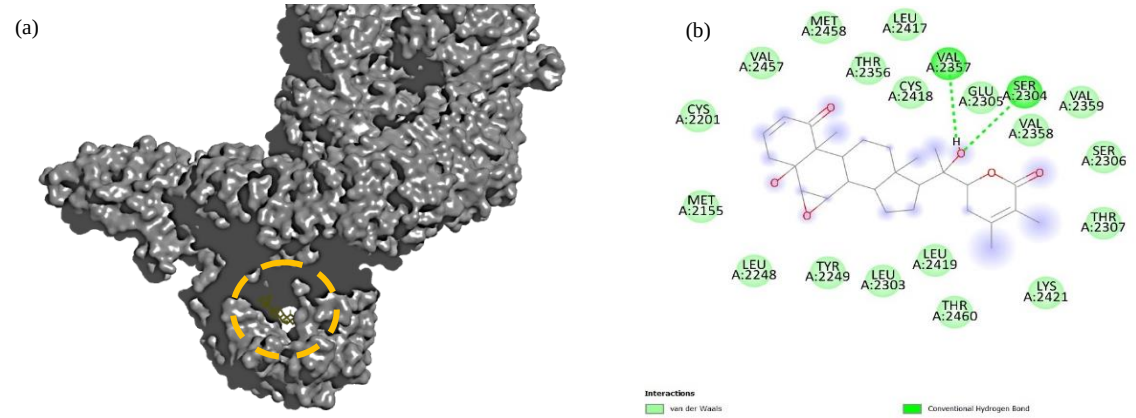

Fig 3.5 (a) The three-dimensional representation depicting the binding mode of Withanolide A with LRRK2 G2019S. (b) The two-dimensional illustration showcasing the binding pattern of the proposed phytochemical, Withanolide A, with the LRRK2 G2019S protein.

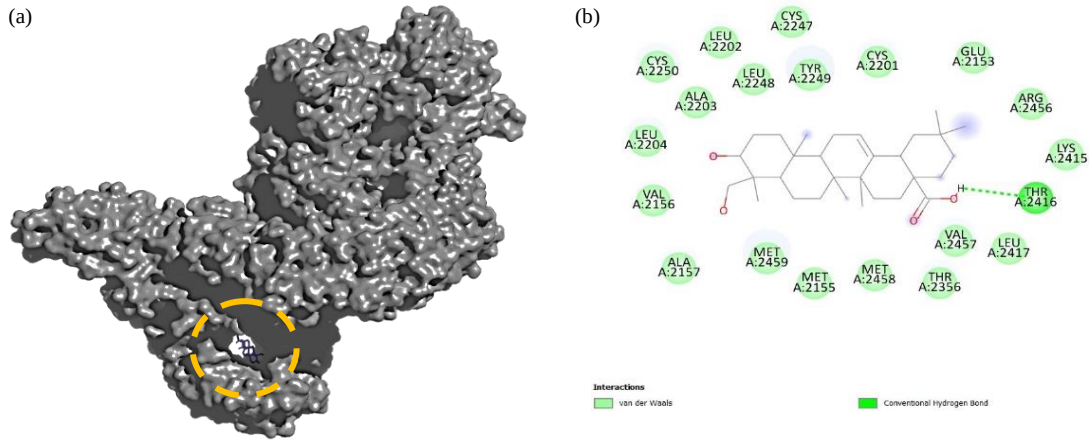

Fig 3.6 (a) The three-dimensional representation depicting the binding mode of Hederagenin with LRRK2 G2019S. (b) The two-dimensional illustration showcasing the binding pattern of the proposed phytochemical, Hederagenin, with the LRRK2 G2019S protein.

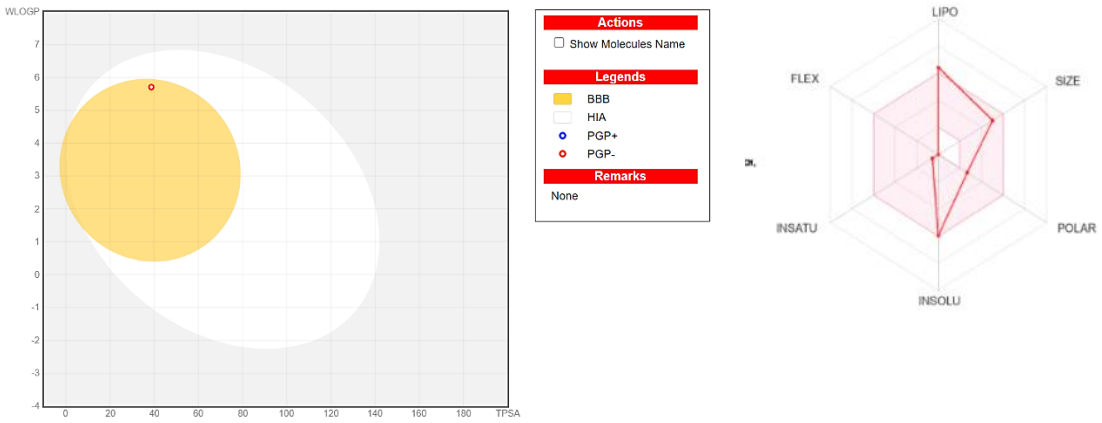

Fig 3.7. The structural depiction of Yamogenin, presented in a boiled egg model, provides a concise yet informative visualization of its molecular configuration

DISCUSSION

LRRK2 G2019S which offers an exclusive opportunity to address multiple pathways in Parkinson's development. Abnormal LRRK2 activity affects mitochondrial function, autophagy, and neurological inflammation. This study targets this LRRK2 G2019S and involves two investigations: one focusing on phytocompounds and the other on FDA-approved drugs. For the first study, Serotobenine, a novel phenolic amide isolated from safflower seeds, served as a reference phytochemical compound in the identification of potential lead compounds. In particular, Serotobenine forms stable bonds and interactions with receptor proteins, boosting its potential as a pharmacological agent. Reference compound scored -8.9 kcal/mol binding and -8.5 kcal/mol docking with CB-Dock2. A docking threshold of -9.0 or lower, was established to identify natural LRRK2 G2019S inhibitors.

Table 3.1 shows the phytochemicals having high BBB permeability within binding affinity scores within the threshold range. Out of the top 5 phytocompounds, Yamogenin, IMPPAT ID IMPHY003711 has the most negative binding energy score. Yamogenin is the phytocompound derived from the shoot of Asparagus officinalis plant belonging to the class of steroids based on chemical classification. Yamogenin was found to possess anti-diabetic, anti-cancer, anti-fungal, antimicrobial and neuroprotective activity. Withametelin (IMPHY003277), Withametelin B (IMPHY009120) and Isowithametelin (IMPHY010687) are phytochemicals extracted from the leaves of the Datura metel plant, classified as steroids based on their chemical structure. These have been identified to exhibit antifungal, anti-proliferative, anti-inflammatory, antioxidant, antipyretic, and neuroprotective properties. Diosgenin (IMPHY003681) is also a steroid derived from the leaves of Asparagus racemosus and possesses antioxidant, antidiabetic and immunomodulatory properties. Further docking validation using CB-Dock2 confirmed these compounds as effective inhibitors of the target protein as shown in Tables 3.5.

Table 3.2 highlights the phytochemicals that are targeting α-synuclein aggregation with their binding affinity score within the threshold range. Out of the top 2 having high GI absorption was considered further. Withanolide A (IMPHY000090) is a steroid derived from Withania somnifera that possesses anticancer, anti-inflammatory, antioxidant, and antimicrobial properties. The neuroprotective advantages of this natural substance include its ability to hinder amyloid formation, reduce α-synuclein clustering, and provide neuroprotection by regulating neural mediators such as acetylcholine [202].

Hederagenin (IMPHY007224) is a Terpenoids sourced from Hedera helix and is an autophagic enhancers that endorse the dilapidation of neurodegenerative mutant disease proteins in vitro, has anti-depressant, anti-inflammatory, anti-cancer, anti-diabetes, anti-inflammation, and anti-oxidation properties [203].

In another study involving FDA-approved drugs, venetoclax was used as a reference. This small molecule is a Bcl-2 inhibitor that was permitted for the treatment of CML in 2016 and AML in 2018 [204]. FDA-approved drugs retrieved from DrugBank, along with a reference drug, were subjected to docking. Venetoclax scored -8.0 kcal/mol in docking; inhibitors required > -9.0 kcal/mol. Those meeting the threshold underwent BBB analysis, resulting in 5 compounds. The top three— Ponatinib, Mosapramine and Drospirenone were selected for further analysis. Further docking validation using CB-Dock2 confirmed these compounds as effective inhibitors of the target protein.

Tables 3.7, 3.8 & 3.9 outline several physicochemical properties of the lead compounds, which play vital functions in determining both the pharmacodynamic and pharmacokinetic processes of drugs. The evaluation of drug likeness heavily relies on the physicochemical characteristics of lead compounds. Among these characteristics, molecular weight holds particular importance, ideally $\leq$ 500 as per Lipinski's rule of five. Additionally, adherence to this regulation entails limiting H-bond donors < 5, H-bond acceptors > 10..

Lipophilicity, indicated through the partition coefficient, should be below 5. Another critical factor affecting a molecule's bioavailability is its topological polar surface area (TPSA), which should ideally be under 140 $Å^2$ to improve oral bioavailability. In this study, the consensus Log Po/w value, averaged from predictions by five different models in Swiss ADME, was used to assess lipophilicity. Considering these factors, the lead compounds demonstrate excellent physicochemical properties.

The prediction of gastrointestinal absorption and blood-brain barrier permeability is a fundamental initial assessment in elucidating the pharmacokinetics and biodistribution of a drug candidate within an organism's biological system. Tables 3.3 & 3.4 represent the drug-likeness and pharmacokinetics of the selected compounds. The study highlights that all lead compounds display moderate water solubility and high gastrointestinal absorption while conforming to Lipinski's rule with minimal violations. Additionally, it underscores the essential need for all drugs to be penetrable to the BBB, as impermeability greatly impedes the treatment of neural disorders. The enzymes CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, which are part of the Cytochrome P450 family, are crucial in metabolizing a wide range of drugs. Inhibition

of these enzymes can lead to significant drug toxicity, highlighting their importance in pharmacokinetics. Notably, all the compounds in this study were found to be non-inhibitors of these enzymes.

Table 3.9 illustrates the pharmacological profiles of leading FDA-approved drugs. Ponatinib exhibits better physicochemical properties than Mosapramine and Drospirenone, all of them possess high molecular weights, with solubility higher than Ponatinib beyond the threshold. In terms of drug-likeness and pharmacokinetics, Ponatinib, Mosapramine and Drospirenone conform to Lipinski's rule of five, with a bioavailability score of 0.55. Drospirenone are effective cytochrome P450 enzyme inhibitors but exhibit poor solubility, whereas show moderate solubility.

In Table 3.10, PkCSM was employed to predict AMES toxicity, oral rat acute toxicity (LD50), and the maximum tolerated dose (MTD) in humans. AMES mutagenicity testing involves utilizing bacterial strains to evaluate the mutagenic potential of a compound on DNA, while the MTD in humans determines the highest dose that can be administered without significant adverse effects. A drug candidate exhibiting an unacceptable toxicity profile would typically not advance to subsequent stages of the drug discovery pipeline, although it is crucial to consider concentration/dosage-dependent toxicity. The oral rat acute toxicity (LD50) estimation provides insights into the lethal dose required to achieve 50% mortality in the test animal cohort, elucidating the relative toxicity profile of the selected compounds. CarcinoPred-EL was utilized to foresee the carcinogenicity of the particular compounds, and none of the phytochemicals demonstrated carcinogenic potential. In both studies, we investigated the potential of phytocompounds, recognized as potent LRRK2 inhibitors, targeting the G2019S mutation. This mutation is known to increase kinase activity in Parkinson's disease (PD) patients. We found that as compared to all the FDA-approved drugs that were docked, five of phytocompounds exhibited strong binding affinity with LRRK2, and two phytocompounds showed inhibition for α-synuclein aggregation yielding promising results.

CHAPTER 4

CONCLUSION

PD, a prevailing neurodegenerative disorder categorized by incremental dopaminergic neuronal loss in the substantia nigra, remains a significant global health concern due to the absence of disease-modifying treatments. Current therapeutic interventions are predominantly symptomatic, addressing motor and non-motor symptoms without halting neurodegeneration. As the second utmost common neurodegenerative disease worldwide.

PD necessitates innovative research approaches to develop neuroprotective and disease-modifying therapies. This study contributes to this endeavor by utilizing an in-silico approach, incorporating molecular docking and ADMET analysis, to identify natural compounds that can inhibit the LRRK2 protein. LRRK2 G2019S mutations linked to familial, sporadic PD, making it a pivotal target for therapeutic intervention. The research focuses on natural phytochemical inhibitors, investigating their potential to interact with this protein with higher specificity and fewer adverse effects compared to synthetic inhibitors or currently available pharmacotherapies. To contribute to these efforts, two complementary studies were conducted using an in-silico approach to identify effective targets and discover novel inhibitors. The first study concentrated on natural inhibitors, while the second explored FDA-approved drugs. Both strategies aimed to target the identified protein more efficiently, ensuring higher safety and fewer side effects compared to other inhibitors.

The research methodology encompasses several critical steps: molecular docking simulations to predict the binding affinity, ADMET analysis, and toxicity and carcinogenicity profiling to investigate the potential of phytochemicals from Indian medicinal plants and currently known FDA-approved drugs. The results indicate that both the phytocompounds and FDA-approved drugs effectively inhibit LRRK2 G2019S. The study examines various phytochemicals derived from Indian medicinal plants,known for their therapeutic properties. The results indicate that these phytochemicals effectively inhibit the LRRK2 protein, a main contestant in the

pathogenesis of PD. The identified compounds include Luteoxanthin, Isowithametelin, Withametelin, Withametelin B, Diosgenin, Daturilinol, Sarsasapogenin, Yamogenin, Daturametelin D, and Datumetelin. These compounds exhibit the capability to cross the BBB, thereby enhancing their potential efficacy in targeting CNS pathology. Furthermore, the study identifies phytochemicals that inhibit α-synuclein aggregation, another critical pathological hallmark of PD. Compounds such as Withanolide A and Hederagenin demonstrate high GI, making them suitable for oral administration targeting α-synuclein aggregation in the gut. The comprehensive analysis of these phytochemicals includes their physicochemical properties, drug-likeness, pharmacokinetics, toxicity, and carcinogenicity profiles. Evaluation suggests compounds are promising for drug discovery and development.

Among the identified compounds, Yamogenin and Withametelin exhibit the highest binding affinity scores with the LRRK2 protein and demonstrate the ability to cross the BBB, making them particularly promising for therapeutic application. Withanolide A and Hederagenin, with high GI absorption, also present significant potential for effective oral delivery targeting α-synuclein aggregation in the gut. These findings suggest that these phytochemical compounds could serve as potent multi-target inhibitors for LRRK2, offering innovative therapeutic avenues for Parkinson's disease. The amalgamation of these two potential targets, LRRK2 in the brain and α-synuclein Gut findings propose plant extracts halt α-syn aggregation, aiding neuroprotection which might delay the disease progression.

For FDA-approved drugs, Ponatinib, Mosapramine and Drospirenone were identified as effective candidates with superior docking scores compared to selected phytocompounds. The capability of these compounds to cross the blood-brain barrier (BBB) could enhance their therapeutic effectiveness. A comprehensive analysis of physicochemical properties, drug-likeness, and pharmacokinetics revealed that phytocompounds generally performed better than FDA-approved drugs.

While the in-silico results are promising, they necessitate further validation through in-vitro assays, such as cell-based assays to assess cytotoxicity, and in-vivo studies in animal models to confirm their efficacy and safety. These subsequent studies are crucial for advancing these compounds from computational predictions to practical applications in clinical settings. The decisive goal is to develop innovative therapies that can deliberate down or halt the progression of PD, thereby significantly improving the quality of life for patients globally.

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Bioinformatics-Guided Drug Repurposing for Precision Management of Parkinson's Disease: Targeting the LRRK2 G2019S

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***Abstract*—Parkinson's disease affects over 2-3% of individuals aged 65 and above, with Leucine-Rich Repeat Kinase 2 (LRRK2) playing a fundamental role in its pathogenesis. The G2019S mutation in LRRK2 upsurges kinase activity, targeting molecules concomitant with apoptosis, and is a mutual genetic cause. LRRK2's involvement in various neurodegenerative diseases and cancer highlights its probable as a therapeutic target. Abnormal LRRK2 activity affects mitochondrial function, autophagy, and neurological inflammation. Targeting LRRK2 offers an exclusive opportunity to address multiple pathways in Parkinson's development. Screening 3,674 FDA-approved drugs for their affinity to LRRK2 identified Ponatinib, a tyrosine kinase inhibitor initially developed for leukemia treatment, as a favourable candidate. Ponatinib's ability to modify LRRK2 activity and potentially offer neuroprotection makes it an attractive option for Parkinson's prophylaxis. Physicochemical analysis via SWISS-ADME supports Ponatinib's suitability. This analysis suggests Ponatinib's repositioning as a PD prophylactic by targeting LRRK2. Further studies are necessitated to investigate its efficacy in preventing Parkinson's disease.**

Keywords—*LRRK2, Parkinson's Disease, Ponatinib, G2019S, Molecular Docking, Drug re-purposing*

I. INTRODUCTION

Parkinson's disease (PD) endorses as a neurodegenerative syndrome categorized by the gradual degeneration of dopaminergic neurons within the substantia nigra, a central cerebral area integral to the regulation of motor functions. The second most predominant neurological illness, Parkinson's disease affects 2-3% of those over the age of 65. Parkinson's disease is categorized by the formation of intracellular inclusions involving the accumulation of α-synuclein aggregates, leading to neurodegeneration within the substantia nigra. The resultant inadequacy of dopamine in the striatum accentuates the central role. Due to its intricacy and multifaceted nature, environmental variables may also contribute to its occurrence in addition to clear hereditary vulnerabilities. Parkinson's disease (PD) manifests primarily due to the selective degeneration of dopaminergic neurons in the substantia nigra pars compacta, coupled with the accumulation of intracellular protein clusters known as Lewy bodies.[1]

LRRK2 gene encodes a considerable 2,527 amino acid protein known as dardarin, categorized by a complex structural conformation. This protein consists of fifty-one (51) coding exonic regions and incorporates innumerable functional domains, including numerous LRRs, an ROC coupled with its COR, a protein kinase domain, and a WD40 motif. The dardarin protein structure integrates these discrete elements, revealing its multi-domain architecture and highlighting the intricate molecular framework of LRRK2 [2], [3]. Funayama and colleagues localized The genomic exploration within a substantial Japanese family of civilians to the Sagamihara family has efficaciously mapped the LRRK2 locus, known as PARK8, to the chromosomal region 12p11.2-q13.1. This consequence stems from a comprehensive genome-wide linkage study, unveiling crucial revelations regarding the genetic foundations of Parkinson's disease within this particular regiment, shedding light on the intricate genetic determinants operative in this population subgroup. Approximately 1–5% of cases of sporadic Parkinson's disease (PD) and 5–13% of familial PD exhibit these particular genetic variations [4]. These are caused by mutations in LRRK2. PD has been linked to a mere few of the more than 100 LRRK2 mutations that have been identified thus far. Included in this category are genetic mutations, on the p arm which contribute to the spectrum of variations associated with this neurodegenerative disease [5].

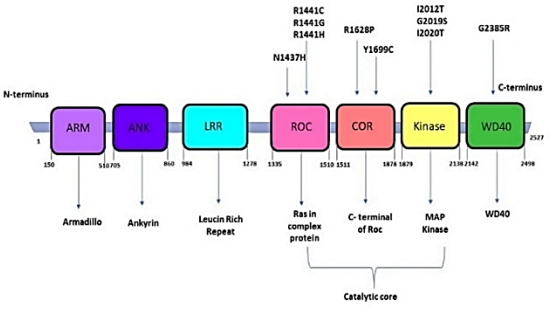


Figure 1. Schematic diagram of functional domains of LRRK2 and pathogenic mutation

A. LRRK2: Unraveling Pathogenesis

The meticulous scientific inquiry into the etiology of Parkinson's disease has extensively explored the participation

of LRRK2, a multifaceted protein manifesting dual functionalities as both a kinase and a GTPase. These mutations affect various cellular pathways, leading to neuronal degeneration[6]

The G2019S mutation, specifically, has been demonstrated to augment the enzymatic activity of LRRK2 kinase, potentially resulting in modified phosphorylation patterns of LRRK2 substrates and contributing to pathological consequences. The precise substrates of LRRK2 enzymatic actions responsible for neuronal demise remain incompletely elucidated. Nonetheless, it is established that both the functional kinase and GTPase activities are imperative for the induction of cellular apoptosis by mutant variants of LRRK2 [7]. Additional exploration is required to meticulously explicate the intricacies of the signaling pathways governed by LRRK2 that endure dysregulation in pathological statuses. The plausible pathogenic pathways involving LRRK2 in Parkinson's disease encompass α-synuclein aggregation, tau pathology, inflammatory responses, elevated oxidative burden, perturbed mitochondrial homeostasis, synaptic disturbances, and aberrations in the autophagy-lysosomal pathway regulatory mechanisms [8].

B. LRRK2 Mutations: Linking PD Pathways

Parkinson's disease is linked to both inherited and spontaneous manifestations when it comes to the multi-domain Ser/Thr kinase LRRK2. An essential regulatory region known as ROC-COR, attached to the ensuing kinase domain, is accompanied by a myriad of mutations linked to various diseases. These mutations lead to discriminating catalytic activity. Ankyrin repeats (ANKs) are identified after armadillo repeat motifs (ARMs) in the N-terminus of human LRRK2 [1].

LRRK2 serves as an MKKK. It orchestrates the phosphorylation of MKK4/7 and MKK3/6, consequently initiating the activation of the c-Jun N-terminal kinase and p38 mitogen-activated protein kinase signaling cascades, respectively. This intricately coordinated molecular cascade assumes a central regulatory role in modulating the cellular stress response [9]. LRRK2 mutations adhere to an autosomal dominant mode of inheritance, characterized by stochastic penetrance, underscoring the nuanced expression of phenotypic traits across individuals. The dominant genetic transmission can arise from either a "gain of function" or haploinsufficiency-induced loss of function. The G2019S and I2020T mutations lead to heightened kinase activity, substantiating a gain-of-function paradigm. These mutations impact the catalytic site of the kinase, influencing substrate access and potentially augmenting substrate interaction. Elevated expression of mutated forms of LRRK2, namely G2019S, R1441C, and Y1699C, resulted in diminished cellular viability in SHSY5Y cells and induced degeneration in transfected primary cortical neurons of mice, while the wild-type counterpart exhibited no such effects [10], [11].

The downstream effects of heightened LRRK2 kinase activity, such as lysosomal dysfunction [12] resulting in lysosomal exocytosis, may present alternative biomarkers associated with LRRK2 activity. One such biomarker is lysophosphatidic acid (also known as BMP [bis(monoacylglycerol) phosphate]), a phospholipid found within late endosomes/lysosomes. Its levels are elevated in the urine of individuals with G2019S-LRRK2 Parkinson's disease compared to healthy controls. Currently, it is being utilized as a biomarker in clinical trials involving LRRK2 kinase inhibitors [13], [14], [15].

C. PyRx

In the quest for lead compounds possessing the necessary biological activity, virtual molecular screening is employed to dock small-molecule libraries onto a macromolecule. This method involves computational techniques to identify and prioritize potential candidates based on their interaction with the target biomolecule. The applicability of this in silico technology to computer-aided drug design is widely recognized. PyRx stands as an open-source software application featuring a user-friendly interface compatible. Its functionality extends to the execution of small-molecule virtual screening through molecular docking processes. Written in the Python programming language, PyRx is compatible with almost all current computers, ranging from desktop computers to supercomputers. Leveraging either a singular workstation or a cluster, Python Prescription (PyRx) demonstrates the capability to process workloads seamlessly, regardless of the underlying machine architecture. The validation of computational binding predictions was conducted through a rigorous comparison of PyRx outputs with X-ray structures. PyRx can be freely downloaded as an open-access software, distributed the Simplified BSD License. Interested users can access the software at http://pyrx.sourceforge.net/downloads.

II. METHOD

A. Protein preparation

The cohort of the LRRK2 protein is facilitated using Discovery-Studio 2024 client. The 3D structure of LRRK2, specifically its Hydrolase Transferase configuration, was retrieved in .sdf format from PubChem. Subsequent to the removal of extraneous ligand and water molecules, the refined protein structure was stored as a .pdb file. Using PyMOL Win, LRRK2 was modified by removing unnecessary water molecules and filling the gaps in chains A and B with a UniProt sequence. The protein that was produced was stored as a.pdb file. After that, PyRx is used to import the LRRK2 .pdb file and convert it to a macromolecule.

B. Ligand preparation

For identifying a potential inhibitory drug for LRRK2, an FDA-approved drug library containing 3,674 drugs is used for docking purposes. The ligands are converted from .sdf to PDBQT format using Open Babel within PyRx. The ligands were opened in Open Babel within PyRx for conversion from .sdf to .pdbqt format after energy minimization.

C. Protein-Ligand docking

PyRx is a virtual screening tool that may be used to assess compound libraries. It makes use of a large number of well-known open-source applications. For the current work, Open Babel is utilized for ligand import and energy reduction, and Auto Dock Vina for docking. Following preparation, the protein and ligand will both be visible in the Auto dock Tab. Molecular docking was conducted employing the Auto Dock Vina program within the PyRx platform. Blind docking procedures were executed utilizing the Vina methods integrated into PyRx.

$$V = W_{vdw}\sum_{i,j}\left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}}\right) + W_{hbond}\sum_{i,j}E(t)\left(\frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}}\right) + W_{elec}\sum_{i,j}\frac{q_i q_j}{\varepsilon(r_{ij})r_{ij}} + W_{sol}\sum_{i,j}(S_i V_j + S_j S_i)\, e^{\left(-\frac{r_{ij}^2}{2\sigma^2}\right)}$$

Equation (1) is an Extended force-field-based scoring function from Auto Dock. The energy between two atoms, i and j, is determined by adding together the contributions from van der Waals forces, hydrogen bonds, and Coulombic interactions, adjusting for empirical free energy with a weight factor.

D. Analyzing Protein-Ligand Interactions

After docking was completed, the results were stored as CSV and output files. The interactions were analyzed using the Discovery Studio Visualizer and Pymol. Compounds exhibiting a Root-Mean-Square-Deviation (RMSD) of inferior to 1 Å, binding free energy lower than -10.0 kcal/mol underwent ranking and evaluation for blood-brain barrier permeability through the utilisation of an online predictor (https://cbligand.org/BBB/predictor.php/). Out of a pool of 3,674 pharmaceutical compounds subjected to testing, only 16 exhibited permeability across the blood-brain barrier (BBB). The chemical structures in the Structure Data Format (SDF) of these BBB-permeable drugs are procured from PubChem.

E. ADME Analysis

The ADME analysis, encompassing Absorption, Distribution, Metabolism, and Excretion, was conducted on a set of five selected drug compounds using the SWISS-ADME framework. This computational tool facilitated an in-depth examination of the pharmacokinetic properties, aiding in the assessment of how these compounds are absorbed, distributed throughout the body, metabolized, and ultimately eliminated. The study examined pivotal determinants, encompassing pharmacokinetics, probability, gastrointestinal absorption, blood-brain barrier permeability, status as a P-glycoprotein substrate, adherence to Lipinski's Rule, violations thereof, aqueous solubility, lipophilicity, and bioavailability. Lipinski's Rule of 5 constitutes a set of parameters employed to evaluate the drug-likeness of a molecule. For oral bioavailability, a compound is considered favourable if it adheres to specific parameters: molecular mass below 500 Daltons, a scarcity of five or fewer hydrogen bond-donating sites, a reduction in hydrogen bond-accepting entities to less than ten, and a calculated logarithm of the partition coefficient (LogP) comprise the criteria under consideration value not exceeding five. These criteria serve as metrics to evaluate the likelihood of a chemical possessing optimal pharmacokinetic attributes for efficient oral delivery.

III. RESULT AND DISCUSSION

Among the 3,674 compounds scrutinized, only 16 demonstrated a Root-Mean-Square-Deviation (RMSD) of inferior to 1Å, binding energy below -10.0 (kcal/mol), meeting the requisite parameters. Ponatinib emerged as the unequivocal victor, exhibiting a binding energy of -10 kcal/mol for the LRRK2 mutant with an RMSD of 0.0Å, thereby surpassing its counterparts in the assessment of molecular interactions.

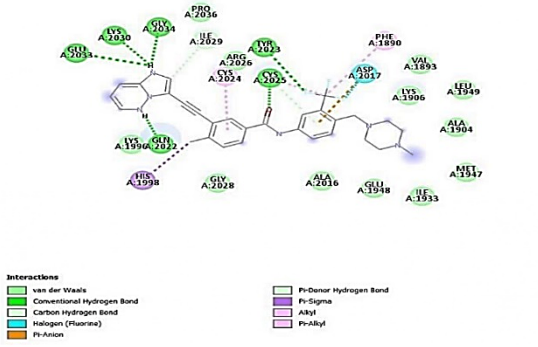
Ponatinib (IC_{50} 0.37nM and 2nM) [16] sold under the brand name *Iclusig,* classified as a BCR-ABL tyr kinase inhibitor, is employed in the therapeutic management of Chronic-phase Myeloid Leukemia (CML) or Philadelphia chromosome-positive Acute Lymphoblastic Leukemia (Ph+ALL). It is specifically indicated for cases that demonstrate resistance or intolerance to previous tyrosine kinase inhibitor therapy. It has a higher docking score than other LRRK2 inhibitors posing a higher number of H-bonds and non-polar interaction, indicating stronger interactions with LRRK2 and a lower toxicity to cells.

A. LRRK2 Inhibitor Binding Unveiled

In the realm of computational drug design, it is imperative to comprehend the physical interactions between proteins and ligands, as this understanding forms the foundational basis for rational drug discovery processes. Evaluate the binding affinities of the inquiry ligands with the protein to determine their molecular interactions., we initiate molecular docking simulations involving the reference drug Venetoclax and LRRK2, followed by an in-depth analysis of their interactions. Subsequently, we scrutinize and present the analysis of the ligand binding site on LRRK2. Venetoclax interacted with LRRK2 and positioned itself in the catalytic core consisting of Asp2017, Ser1954, Cys2025, and Lys1996.

We observed favourable docking scores for five compounds—Ponatinib, Mosapramine, Drospirenone, Alectinib, and Algestone Acetophenide. Notably, these compounds exhibit blood-brain barrier (BBB) permeability and interact with Leucine-Rich Repeat Kinase 2 (LRRK2) through crucial catalytic residues. Two compounds, namely Algestone Acetophenide and Drospirenone, displayed higher docking scores but lacked significant interactions, rendering them suitable for exclusion from further analysis. Analysis of hydrogen bond interactions revealed that Ponatinib establishes six hydrogen bonds with LRRK2, while Mosapramine and Alectinib form one and three hydrogen bonds, respectively. Hydrogen bonds play a pivotal role in modulating ligand binding specificity, and their substantial impact is explicitly incorporated into GRID, a computational methodology designed to identify energetically favourable ligand binding sites on a target molecule with a known structure. The strength and orientation of these bonds are crucial factors influencing the overall bonding interactions [17].

The proposed drug and reference drug shared common interacting residues, specifically Glu2033, Cys2025, Ala1904, and Gly2028, forming four bonds. The 2D and 3D interactions of the compounds ponatinib and venetoclax with the LRRK2 protein are delineated in the provided data.



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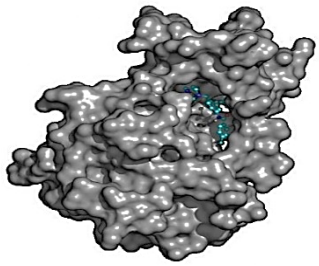
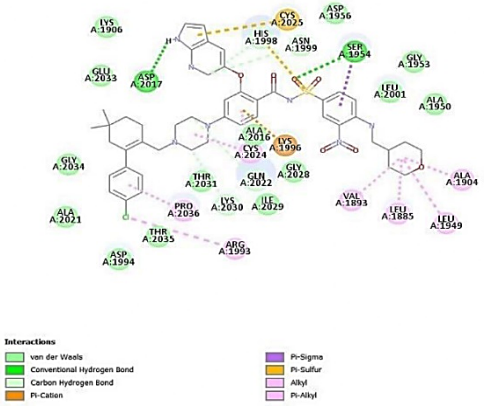
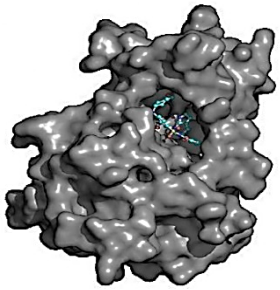


(b)

Figure 2. (a) The two-dimensional representation depicting the binding mode of Ponatinib with LRRK2 G2019S. (b) The three-dimensional illustration showcasing the binding pattern of the proposed pharmaceutical agent, Ponatinib, with the LRRK2 G2019S protein.



(a)



(b)

Figure 3. (a) Two-dimensional depiction of the binding mode between Venetoclax and LRRK2 G2019S. (b) Three-dimensional representation illustrating the binding pattern of the reference drug Venetoclax with LRRK2 G2019S.

B. PPIs between LRRK2 Receptor and Genes Underlying Parkinson's Disease

The protein-protein interaction (PPI) analysis involving LRRK2 and other genes associated with Parkinson's disease (PD) revealed several noteworthy interactions. Employing statistical analysis on the STRING dataset, the p-value for PPI enrichment was determined to be 3.22e-15, signifying a high degree of significance. The protein dataset demonstrated a notable average local clustering coefficient of 0.708, signifying a substantial degree of interconnectivity among the proteins. The edges in the network represent physical interactions between proteins. Pink and light blue lines denote established interactions, while green, red, and navy blue lines signify predicted interactions. Yellow, white, and black lines represent additional interactions. The network encompassed 21 nodes and 97 edges, underscoring the complexity of the interactions. These findings underscore the substantial and meaningful connections among the PD-associated genes. The PPI network database facilitates the exploration of correlations between the physical and functional properties of proteins, providing valuable insights into the intricate relationships within the molecular landscape of Parkinson's disease.

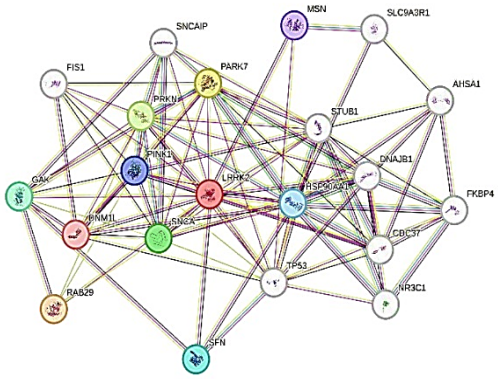


Figure 4. PPI network involving 21 interconnected genes.

C. ADME analysis of Ponatinib

ADME analysis uses Swiss ADME to identify a drug's physiochemical and ADME characteristics. The data supplied encompasses crucial physiochemical attributes, including Molecular Weight and rotatable bond count, along with hydrogen bond acceptor (HBA) and donor (HBD) quantities. along with the assessment of topological polar surface area (TPSA). The ADME i.e. Absorption, Distribution, Metabolism, and Excretion evaluation of therapeutic compound yielded affirmative pharmacokinetic findings, affirming its favourable characteristics for further development and potential clinical application. The drug had significantly higher GI absorption and improved penetration of the brain's endothelial tight connections. We discovered that the physiochemical property values for ponatinib were

within acceptable limits. The Lipinski rule of five is used to anticipate the physical and chemical features of a bioactive molecule that will be orally bioavailable. The drug was identified in accordance with Lipinski's rule of five, demonstrating adherence to key physicochemical parameters essential for optimal oral bioavailability and absorption. Lipophilicity is another important characteristic that is assessed using publicly accessible prediction models in SwissADME. The drug's lipophilicity, quantified as 4.11 using the log value of the partition coefficient between given Octanol and Water (Log Po/w or XLOGP3), was determined. The bioavailability score, denoted as 0.55, was assessed. Additionally, the drug exhibited elevated skin permeability, with a measured value of Log-6.63 cm/s for the permeability coefficient (Kp). The consensus partition coefficient value (Log Po/w) for ponatinib was 4.30, indicating moderate solubility. Illustrates the pharmacological profile through a visual representation analogous to the molecular structure of the drug, resembling the boiled egg image.

IV. CONCLUSION

The top 5 compounds with the greatest binding affinities to LRRK2 bind to one or both of the key inhibitory sites, Glu2033, Cys2025, Ala1904, and Gly2028. Docking yielded one drug with maximum inhibition and higher binding affinity than the reference drug with BBB permeability. The ADME research shows that Ponatinib is the most efficient and gives the best results with a higher number of H-bonds and non-polar interactions. The other parameter values indicate good solubility, physicochemical properties, and bioavailability for Ponatinib, and they are suitable with significant values. LRRK2 participates in diverse cellular pathways, spanning multiple subcellular compartments. Consequently, mutations in its structural composition exhibit proapoptotic effects and are frequently associated with lethality. Hence, an imperative need arises for a potent LRRK2 inhibitor capable of effectively suppressing its aberrant kinase activity, underscoring the pressing demand for targeted pharmacological interventions in this context.

TABLE I. MOLECULAR DOCKING ANALYSIS: COMPOUND BINDING AFFINITIES, HYDROGEN BONDING, AND INTERACTIONS.

S.No.	Compound	Docking Score (kcal/mol)	Types of interaction	
			H-bond with distance (in Å)	Non-polar interactions
1.	Ponatinib	-10	GLU2033, LYS2030, GLY2034, TYR2023, CYS025, GLN022	PRO2036, ILE2029, ARG2026, CYS2024, PHE1890, ASP2017, VAL1893, LYS1906, LEU1949, ALA1904, MET1947, ILE1933, GLU1948, GLU1948, ALA2016, GLY2028, LYS1996, HIS1998
2.	Mosapramine	-9.8	ARG2026	GLU1948, ALA1950, LEU1949, ALA1904, LEU1885, VAL1893, PHE1890, LYS1906, TYR2023, ASP2017, GLN2022, CYS2025, HIS1998, LYS1996, CYS2024, GLY2028, MET2027, ALA2016, ILE1933, LEU2001
3.	Drospirenone	-9.7	-	LYS2112, ILE2111, LEU2115, LEU2092, GLY2090, LYS2091, ASP2094, PRO2093, LYS2109, GLU2108, PRO2095, TYR2064
4.	Alectinib	-9.6	LYS1996, ILE2029, ASP2017	LYS2030, HIS1998, ASN1999, GLN2022, LEU1949, ALA1950, ALA1904, ALA2016, LEU2001, GLU1948, ILE1933, LYS1906, MET1947, VAL1893, LEU1885, CYS2025, ARG2026, GLY2028
5.	Algestone Acetophenide	-9.3	-	LYS1906, GLU1948, LEU1949, ALA1904, CYS2025, CYS2024, LYS1996, GLN2022, ASN1999, HIS1998, SER1954, GLY1953, LEU2001, LEU1885, ALA1950, MET1947, ILE1933, ALA2016, ASP2017

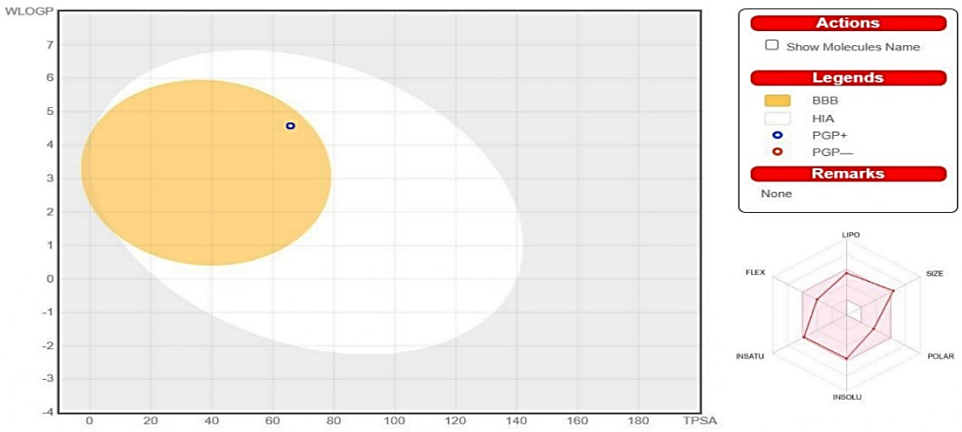


Figure 5. The structural depiction of Ponatinib, presented in a boiled egg model, provides a concise yet informative visualization of its molecular configuration.

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Department of Toxicology, School of Chemical and Life Sciences, Jamia Hamdard, New Delhi

Certificate Of Participation

This is to certify that Prof./Dr./Ms./Mr. Parneet Kaur
of Delhi Technological University has Participate as ***Delegate*** in the Two Days National Symposium on ***"Recent Advances in Neurochemistry and Neurosciences"*** held from 25th April 2024 to 26th April 2024 at Convention Centre, Jamia Hamdard, New Delhi. He/She has also presented in ~~Young Investigator~~/Poster Session.

Prof. Mohammad Akram
Organizing Secretary

Prof. Suhel Parvez
Organizing Chairperson

Prof. Prakash Babu Phanithi
Secretary General (HQ), SNCI

JAMIA HAMDARD
(Deemed to be University)
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One Week STUTI Workshop
Translational Neuroscience: Bridging Gap between Bench to Bedside

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This is to certify that Dr./Ms./Mr. Ms. Parneet Kaur
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Prof. Mohammad Akram
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Prof. Prakash Babu Phanithi
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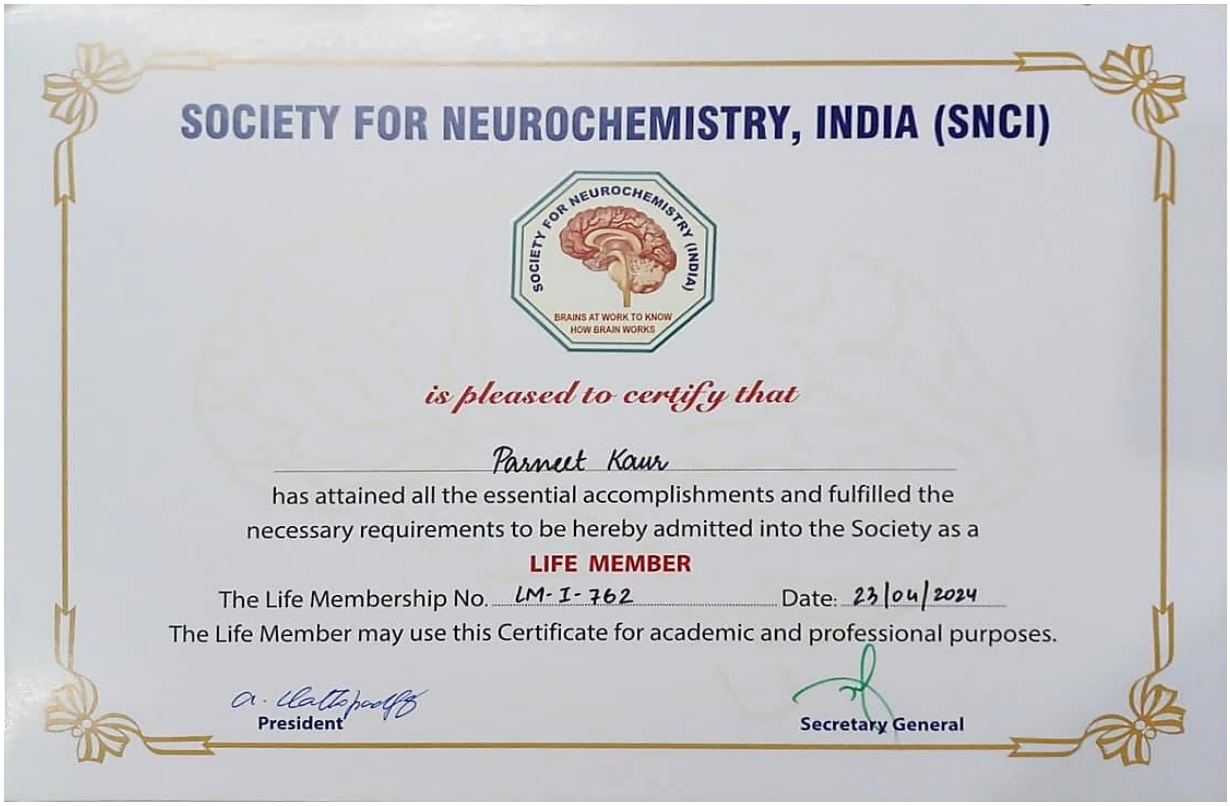

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Curriculum Vitae

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EDUCATION

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CBSE (Class XII)	2019	Poorna Prajna Public School	86 %
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INTERNSHIPS

Online Non-Credit Course Authorized by IBM and offered through Coursera - SQL: A Practical Introduction for Querying Databases Mar 2024-Apr 2024

- Enhanced proficiency in SQL through a comprehensive course, leading to a 30% improvement in query efficiency and data retrieval accuracy; facilitated smoother access to critical information and streamlined decision-making processes within the organization.

Online Non-Credit Course Authorized by IBM and offered through Coursera - Python for Data Science, AI & Development Jan 2024-Mar 2024

- Enhanced data science and AI skills through the completion of an intensive Python course, focusing on statistical analysis, and data visualization techniques, enabling proficient development in dynamic tech environments

Winter Intern, AIIMS, New Delhi Dec 2022-Feb 2023

- Learned basic about coding language R-4.2.2.
- Worked in Bioinformatics lab.

Online Training and Internship in Data Science, Wissenaire-IIT Bhubaneshwar & 1Stop.ai Aug 2023-Oct 2023

- Worked on two Projects - Hate speech detection, Spam SMS Classification of Industrial Program on Data Science from Personifwy.

Hands on Training, CIIDRET, UDSC Feb 2023

- Participated in two weeks of National Workshop on "Immuno-Biology Techniques & Their Applications".

Online Summer Training Program, Shivaji College, University of Delhi June 2021-July 2021

- Participated in four weeks Online Summer Training program on Research Methodology 2.0 organized by the Department of Biochemistry, Shivaji college.

ACADEMIC ACHIEVEMENTS AND AWARDS

- Awarded with silver scholar badges in academic session IX & X

TECHNICAL SKILLS

R Programming, Python, SQL	Proficiency in MS Office suites	Machine learning

POSITIONS OF RESPONSIBILITY

- As head prefect of the schoolhouse
  - Leader of the fellow students
- Member of Biosoc-DTU
  - As a content creator and corporate affairs department
- Member of Anveshan-Biochemistry Society

CONFERENCE

- Attended and Presented a Poster in the 3rd International Conference on "Antimicrobial Resistance, Novel Drug Discovery and Vaccine Development: Challenges and Opportunities" Held on March 18-20, 2024, Organized by SRM University Delhi- NCR, Sonepat.
- Attended and Presented a Poster in the One-week STUTI Workshop on" Translational Neuroscience: Bridging Gap between Bench to Bedside" organized by Jamia Hamdard & SNCI (Society for Neurochemistry India).
- Participated and presenting a paper titled "Computational Navigation of Parkinson's Disease: Bioinformatics-Driven Drug Repurposing Strategies Targeting LRRK2 G2019S" at the 4th International Conference on Advance Computing and Innovative Technologies in Engineering organized by Galgotias College of Engineering and Technology in association with IEEE.

OTHER INFORMATION

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