EXPLORING PHYTOCHEMICALS FOR PARKINSON'S THERAPEUTICS THROUGH IN-SILICO DRUG DISCOVERY AND REPURPOSING TO TARGET VPS35 AND ALPHA-SYNUCLEIN

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

MASTER OF SCIENCE
in
BIOTECHNOLOGY
by

ANJALI ROY
2K22/MSCBIO/10

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

June, 2024

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CANDIDATE'S DECLARATION

I, Anjali Roy, bearing Roll No. 2K22/MSCBIO/10 hereby certify that the work which is being presented in the thesis entitled **"Exploring Phytochemicals for Parkinson's Therapeutics through In-Silico Drug Discovery and Repurposing to Target VPS35 and Alpha-Synuclein"** 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.

Anjali Roy

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 **Anjali Roy** (2K22/MSCBIO/10) has carried out their search work presented in this thesis entitled **Exploring Phytochemicals for Parkinson's Therapeutics through In-Silico Drug Discovery and Repurposing to Target VPS35 and Alpha-Synuclein** or 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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Exploring Phytochemicals for Parkinson's Therapeutics through In-Silico Drug Discovery and Repurposing to Target VPS35 and Alpha-Synuclein

ANJALI ROY

ABSTRACT

Background: PD is a neurodegenerative conditions that is characterized by the loss in the DA neurons in substantia nigra, that causes motor manifestations such as tremors, rigidity, bradykinesia, and postural instability. Recent studies can highlight the role of mutations, particularly in VPS35 gene, for the development of PD. This study aims to identify potential inhibitors of the VPS35(D620N) protein using an in-silico approach. The research focused on investigating the drug-likeness and pharmacokinetics of select phytochemicals with notable BBB permeability and high gastrointestinal (GI) absorption to identify potential therapeutic agents. Initially, a library of 1,580 phytochemicals from various medicinal plants was screened for checking the Lipinski's rule by Swiss ADME tool . Following this, binding interactions were analyzed with Discovery Studio Visualizer, and blind docking simulations using PyRx, with docking parameters calibrated to align with the reference drug's scoring system. Docking scores were validated using CB-Dock2. Toxicity analysis can be done using PkCSM. Swiss ADME was used to predict ADMET properties. Carcinogenicity assessments can be done using CarcinoPred-EL.

Result: Five phytochemicals namely Luteoxanthin, Isowithametelin, Withametelin, Withametelin B, Diosgenin, Daturilinol and Sarsasapogenin derived from the selected medicinal plants show potential as drug candidates for PD. Additionally two more compounds Withanolide A and Hederagenin, can show high GI absorption and thus can be a potential inhibitor against the Vps35(D620N) as act as α-synuclein aggregates inhibitiors in the gut. Findings demonstrate that these compounds can these are ideal candidate for therapeutics in Parkinson’s disease.

Conclusion: Several phytochemical compounds found in medicinal plants have shown promise as potential drug candidates for treating Parkinson's disease associated with the mutant VPS35 (D620N). This potential is due to their demonstrated low binding energy and stability when interacting with the target protein.

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LIST OF ABBREVIATIONS

PD	Parkinson's disease
LBs	Lewy Bodies
SNpc	Substantia nigra pars compacta
GABA	Gamma aminobutyric acid
VPS35	Vacuolar protein sorting 35
TGN	Trans- golgi network
SNX	Sorting nexin
CSC	Cargo- selective complex
CI-MPR	Cation-independent mannose 6-phosphate receptor
ENS	Enteric nervous system
BBB	Blood-brain barrier
IMPPAT 2.0	Indian Medicinal Plants, Phytochemistry, And Therapeutics 2.9
GBA	Gut brain axis
ROS	reactive oxygen species
MUL1	mitochondrial E3 ubiquitin ligase 1
EIF4G1	eukaryotic translation initiation factor 4 gamma 1 gene
BAR	Bin-Amphiphysin-Rvs
PI3P	phosphatidylinositol 3-phosphate
β2AR	β2-adrenergic receptor
GLUT1	glucose transporter 1
GBA	glucocerebrosidase gene
LRRK2	leucine-rich repeat kinase 2
PINK1	PTEN-induced putative kinase 1
SOD2	superoxide dismutase 2
DA	Dopaminergic

CHAPTER 1

Introduction and Literature Review

1.1. Overview

Neurodegeneration is characterized by the gradual deterioration and loss of structure or function of neurons, the specialized cells responsible for transmitting information in the brain and nervous system. This process can result in various neurological disorders, such as AD, PD, HD and ALS, among others. The term "shaking palsy" was introduced by Dr. James Parkinson in 1817 to describe PD, a neurodegenerative conditions marked by both motor and nonmotor indications. The disease significantly impacts muscle control and mobility, affecting patients, families, and caregivers alike. The neuronal loss in the striatum primarily is the cause of motor symptoms while neuronal loss in other regions can cause non-motor symptoms. Bradykinesia, muscular rigidity and resting tremors are the motor characteristics of PD and collectively called as Parkinsonism [1] , [2] . The majority of individuals with Parkinson's disease (PD) are in their 30s and 40s. There is a gender difference, with a 3:1 ratio of males to females, partly due to estrogen's protective effects on the brain's dopaminergic system, leading to a delayed onset in females [3] ,[4].

1.2. Literature Review

1.2.1 Parkinson's Disease

PD is a second most prevalent neurological condition which is characterized by both motor and non-motor indications. It is most prevalent in middle-aged and older adults can affect 1% of individuals over the age of 60 and [5], [6]. PD is marked by symptoms such as bradykinesia, tremors, impaired vision, depression, sleep disturbances, dementia, and cognitive impairments [7]. The degeneration of neurons in the caudate putamen can cause the reduction in dopamine level that is the prime cause for the motor symptoms in PD [8]. Currently, there is no treatments to halt or slow the disease's progression, making it essential to identify and validate new therapeutic drug targets for its management. PD is often an unpredictable condition, likely resulting from complex interactions between aging, environmental exposures, and genetic risk factors [9]. Neuropathologically, reduction DA neurons in SNpc is the characteristics of PD that leads to reduction in dopamine level in the caudate-putamen, and by reactive gliosis [9] . The surviving brainstem neurons contain intracytoplasmic, eosinophilic inclusions called Lewy bodies, that primarily contain fibrillar α-synuclein [10], [11].

PD is a disease that starts in the brainstem and further advances to higher cortical regions in the vagal nerves, as well as the olfactory nucleus [12]. It can impact the extrapyramidal system, encompassing the motor structures within the basal ganglia. It arise due to the loss of dopaminergic activity, leading to reduced motor function and the clinical symptoms of the disease [13] . The degenerated pigmented DA neurons and presence of LBs is the histopathological characteristics of PD [14] , [15]

The motor activity is regulated by excitatory (D1) and inhibitory (D2) dopamine receptors. This system includes the pars reticulata segment of the SNpr and the basal ganglia, encompass the internal GPi of the striatum. These constituents are elements of more extensive circuits found in the cortex and thalamus. The thalamic inhibition and GABA dysfunctioning can occur in PD patients because of dopamine loss in striatum that cause GPi/SNpr circuit to become more active. As a result, the frontal cortex is less able to be activated by the thalamus, which ultimately leads to the reduced motor activity that is indicative of PD [16]. The dopaminergic function is diminished by the gradual deterioration of DA neurons in substantia nigra region [16].

1.2.2 Risk factors associated with Parkinson's disease

The risk Factors for PD include both environmental factors and genetic factors. Gene mutations include alterations in the SNCA, EIF4G1, GBA, LRRK2 gene loci, PINK1 gene loci, and the SOD2 gene [17] , [18] , [19] , [20] , [21] . Exposure to environmental toxins, head injuries, mitochondrial dysfunction, and oxidative stress due to free radicals and neurotoxins. Other factors that can be a potential risk are post-infection states and signal-mediated apoptosis. The formation of protein aggregates, due to overproduction or overexpression, can be influenced by genetic mutations. Such mutations can lead to alpha-synuclein aggregation and the formation of insoluble fibrils associated with Lewy bodies (LBs) [22]. The formation of LBs is associated with the overproduction of misfolded ubiquitin proteins, which are crucial for protein recycling. The dysfunctioning of the protein recycling system can cause the buildup of proteins in body [23], [24]. Lewy bodies formation can contribute to the neurodegeneration, a characteristic of PD. These show distinct lesion patterns at different disease stages, like the early lesions can cause the premotor olfactory and REM sleep features of PD. In later stages, these lesions in the nigrostriatal region can show other common manifestations of the disease [25] , [26].

1.2.3 Symptoms of PD

Motor symptoms of PD include the classic triad of slowed movement, rigidity, and resting tremor. Bradykinesia, marked by slowness and decreased movement

amplitude, affects 80-90% of patients. Resting tremor, a common initial symptom in most of patients, primarily affects the hands and also involve the lips, tongue, jaw, chin and legs typically subsiding with movement or sleep. Rigidity can show resistance to passive movement in relaxed limbs and is often associated with the "cogwheel" phenomenon. Other motor symptoms include postural instability, which increases the risk of falls and injuries in later stages, dysarthria, and dystonia [27], [28] , [29], [30]. Non-motor symptoms can show autonomic dysfunction, sensory, neuropsychiatric symptoms and disturbance in sleep. Additional symptoms are fatigue, weight loss. The autonomic dysfunctioning include constipate, sexual dysfuction, sweating, urinary retention and orthostatic hypotension. The sensory impairment involve olfactory dysfunction. The neuropsychiatric symptoms include anxiety, dementia, depression, panic disorders. Sleep disturbance include insomnia, sleep attacks and sleep apnea [31], [32], [33].

1.2.4 Genetic causes of Parkinson's Disease

Mutations associated with familial PD have been detected in a range of gene. These genes are both autosomal dominant and recessive like SNCA, VPS35 , LRRK. [34]. These genes are known to play roles in mitochondrial function and biogenesis, highlighting the connection between mitochondrial defects and the progression of PD. The involvement of these genetic mutations underscores the potential for mitochondrial dysfunction to act as a significant contributor to the disease, emphasizing the importance of targeting mitochondrial health in developing future PD therapies [35]. In the VPS35 gene a single missense mutation, D620N can causes autosomal dominant, late-onset PD. While other mutations in this gene have been discovered, their pathological significance is still uncertain. VPS35 mutations are rare, and can be seen in very less cases of both familial and sporadic PD cases [36], [37], [38]. Thus, VPS35 became a target for the therapeutics for studying the disease progression mechanism. Currently, there are no therapeutics available that can cure PD. Levodopa remains the primary medication used, but it only slows symptom progression rather than halting or reversing the disease. This creates an urgent need for new therapeutic agents that can both treat the disease and target its progression. The search for such agents can be accelerated through molecular docking techniques and bioinformatics analysis. These methods allow for the identification of potential compounds that are not only effective but also safe, minimizing the risk of side effects on patients' bodies. Developing new therapeutics that can effectively target the underlying mechanisms of PD without causing adverse effects is crucial for improving patient outcomes.

1.3. Structure and role of VPS35 In Retromer

Familial PD is caused by an inheritable mutations in autosomal dominant genes. Familial forms of Parkinson's disease are associated with inherited mutations in at least 15 genes. Only 10% of cases are familial, involving both autosomal dominant and recessive conditions and their inheritance patterns and can be characterized by key motor symptoms [10]. A specific D620N mutation in the gene that encodes VPS35 results in the onset of this form of autosomal dominant, late-onset Parkinson's

disease [39], [40], [41]. PD is primarily idiopathic, with age being the predominant risk factor. In mammals, the SNX dimer is essential for recruiting the trimer protein to endosomes [42]. The mammalian retromer is comprised of a conserved trio of VPS proteins: VPS35, VPS29, and VPS26. The retromer is a hetero-pentameric complex comprising a dimer of SNX and a trimer of VPS26-VPS29-VPS35. In yeast, the SNX dimer consists of VPS5 and VPS17 [43], [44]. The retrograde transport of transmembrane proteins is facilitated by the this multi-subunit complex [45] , [46], [47]. The interactions with the accessory proteins is crucial for associating cytoskeleton, selection of cargo and deformation of membrane. This efficacy hinges the engagement of endosomal membrane, that is orchestrated by specific SNX proteins and GTPases Rab5 and Rab7 [48], [49].

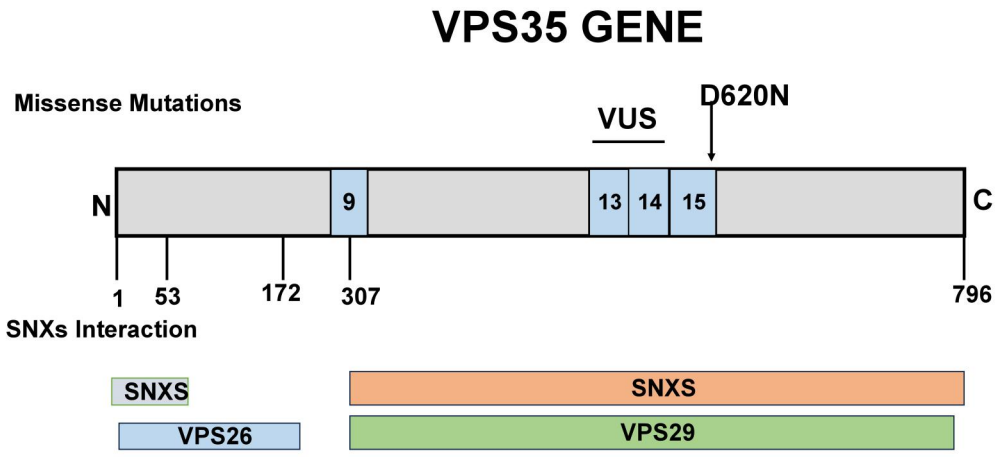

Fig. 1.1 Schematic diagram of VPS35 gene, genetic structure and interactions and the associated D620N mutation.

1.3.1 Vps35 and Retromer functioning

The VPS35 is a fundamental component of , a hetero-pentameric complex [50]. It was initially discovered in yeast, and is a protein complex that connects with the endosome, enabling the transfer and reutilizing of transmembrane protein cargo from the endosome to both the Golgi complex and the plasma membrane [51], [44], [52]. The retromer is conserved across species from yeast to mammals and participates in specific cellular pathways that may contribute to disease processes. It is usually categorized into two parts: CSC trimer and a SNX dimer. The CSC, made up of VPS26, VPS29, and VPS35, is in charge of recognizing and organizing protein cargo [53] , [54] . VPS35, comprising 796 amino acids, serves as the largest component. Structural investigations into VPS35 indicate a notable degree of flexibility, adopting an α-solenoid fold that spans its entirety. This specific fold is crucial for its

interaction with VPS29 and binds to the C-terminal region of VPS35 [55]. The CSC engages with an SNX dimer, typically composed of SNX1 or SNX2 along with SNX5 or SNX6 in mammalian cells (or SNX5 and SNX17 in yeast). These structural features facilitate the retromer's association with the endosomal membrane. The SNX proteins, known for possessing both a BAR domain and a phox homology domain and they are the member of SNX-BAR family [56] . The N-terminal segment of VPS35 attaches to VPS26 via a PRLYL motif [57]. The PX domain binds to PI3P on the membrane [58] , [59] . Upon activation, VPS34 phosphorylates phosphatidylinositol, producing PI3P on the endosomal membrane. This lipid modification then recruits downstream molecules such as RAB7A and SNX proteins [58] . The CSC in isolation, does not possess the capability to establish a strong interaction with the SNX dimer. To overcome this limitation, RAB7A acts as an additional tether, guaranteeing the retromer's adherence to the endosomal membrane [60] . A distinct subset of the CSC exhibits the capacity to engage with diverse proteins, highlighting the retromer's involvement in precise endosomal sorting pathways. This subset can, for instance, associate with the WASH complex, comprising WASH1, FAM21, along with adaptor proteins like SNX27. These interactions facilitate the transfer from the endosome to both TGN and the plasma membrane. [61] , [62] , [63] . The WASH complex is crucial for organizing actin patches on the endosomal membrane, creating specialized regions where cargo molecules are sorted for transport to the TGN or the plasma membrane. It engages with the retromer through the binding of the unstructured tail FAM21 with VPS35 C terminal. This crucial interplay is responsible for retrieving specific cargo, such as the CI-MPR, from the endosome and conveying it to TGN [62] . The WASH complex has the ability to associate with both the retromer and SNX27, enabling the retrieval of particular cargo from the endosome to the plasma membrane. This intricate process includes the transportation of proteins such as the β2AR, GLUT1, and numerous metal ion transporters [51] , [64]. The retromer is acknowledged as a crucial mechanism for conveying membrane proteins to the dendritic extensions of neurons. Studies suggest that the retromer plays a role in delivering membrane proteins to both the post-synaptic compartment and extra synaptic sites (such as the β2AR) along the dendrite [65].

1.3.2 VPS35 Mutation in Parkinson’s

The discovery of missense mutations in VPS35 suggests that impaired retromer complex may play a role in Parkinson's disease. These mechanisms may include a toxic gain-of-function, a dominant-negative effect, or haploinsufficiency. The mutations in this gene as well as the dominant inheritance pattern of PD mutations, suggests that heterozygous protein mutations could act through various mechanisms. Distinguishing among these possibilities to understand the pathological effects of disease-causing mutations is a challenging and intricate endeavor, particularly when multiple mechanisms may be concurrently involved [66].

α-Synuclein is a presynaptic protein involved in the assembly of the SNARE complex and the exocytosis of neurotransmitters [67], [68], [69]. The main problem

with α-synuclein is that it tends to clump together, starting as small, soluble clusters and progressing to larger, insoluble fibres that form Lewy bodies. Although it is mostly present in the cell's cytosol, but can also be found in mitochondria, where it causes dysfunction [70], [71], [72]. New findings indicate that α-synuclein can move from one cell to another through different methods, such as non-classical exocytosis, exosomal release, and nanotubes connecting cells. According to the Braak staging hypothesis, the initial dissemination of misfolded α-synuclein is believed to originate in the olfactory bulb and the gastrointestinal tract. This theory is corroborated by the presence of α-synuclein accumulation in the enteric neurons of individuals in the early stages of PD [73].

1.4. Vps35(D620N) induced Neurodegeneration

Three possible cellular mechanisms have been suggested for how mutant VPS35 might cause neurodegeneration. These mechanisms disturb the cellular physiology of body and thus causes pathology. It is not yet clear if these mechanisms contribute to neurodegeneration or if they are connected. Nonetheless, these investigations offer valuable understanding of the cellular pathways affected by the D620N mutation in VPS35 in neurons [66].

1.4.1 Impaired WASH complex binding and autophagy defects

Studies indicate that in human cell lines the D620N mutation disrupts the binding of the WASH complex with VPS35 [74] , [75]. The D620N mutation in VPS35 reduces its ability to bind effectively to FAM21, a crucial component of the WASH complex. This diminished binding results in the WASH complex being less recruited to endosomes. Research indicates that this reduced interaction between D620N VPS35 and FAM21 disrupts the normal sorting of CI-MPR and GLUT1 proteins within endosomes [76] . It has been found that in heterozygous KO mice lacking VPS35, there was a decrease in LAMP2a levels. This reduction was attributed to increased lysosomal degradation of LAMP2a, as it was not efficiently recycled back to the trans-Golgi network (TGN) [77]. Reduced levels of LAMP2a, likely stemming from impaired chaperone-mediated autophagy (CMA), causes a build-up of α-synuclein in heterozygous VPS35 knockout mice brain. Similarly, the introduction of human D620N variant into the dopaminergic neurons of mice led to decreased LAMP2a levels and subsequent α-synuclein accumulation [78] . This suggest that chaperone-mediated autophagy (CMA) helps regulate the breakdown of α-synuclein in lysosomes. However, experiments in rats involving the overexpression of various human VPS35 variants using AAV did not show any alterations in the levels, phosphorylation, or pathology of α-synuclein in DA neurons [79].

1.4.2 Disrupted AMPA receptor trafficking

The retromer complex is present in various parts of the neuron, such as the cell body, axon, and dendrites. Notably, VPS35 and the retromer complex are localized within dendritic spines, where they engage with and facilitate the trafficking of the AMPA

receptor GluR1 [79], [80], [81].When D620N VPS35 is overexpressed, it does not target dendritic spines as efficiently as the wild-type (WT) protein. This results in impaired trafficking of GluR1, which in turn affects synaptic transmission [82] . Reduced VPS35 function, akin to the impact of the D620N mutation, results in abnormal trafficking of AMPA receptors. Neurons lacking VPS35 exhibit diminished synaptic transmission, likely due to disrupted trafficking of both GluR1 and GluR2. The impaired maturation of dendritic spines can be reversed by increasing the levels of GluR2, but not GluR1, suggesting that the altered trafficking of GluR2 is the main cause of this effect [83]. The D620N mutation interferes with the retromer's ability to correctly sort GluR1/R2 to dendritic spines, impacting both synaptic transmission and morphology [84].

1.4.3 Lysosomal stress

The endo-lysosomal system is involved in α-synuclein toxicity as this protein needs to be accurately transported to the lysosome for degradation. Mistrafficking of alpha-synuclein or its lysosomal enzymes can enhance its toxicity by causing abnormal accumulation. Additionally, this mistrafficking can cause broader lysosomal dysfunction, further exacerbating the accumulation and toxic effects of alpha-synuclein. Proper lysosomal function is crucial to prevent these toxic effects and maintain neuronal health [85] . Dysfunction in the endo-lysosomal system can indirectly influence Parkinson's disease (PD) pathogenesis through its effects on alpha-synuclein. VPS35 is a crucial part of the retromer complex, and its mutation VPS35[D620N] is linked to a disease. This mutation affects the trafficking process that relies on the retromer. Despite appearing simple, the molecular impact of this Parkinson's disease-related mutation is relatively minor. It specifically interferes with the interplay between VPS35 and FAM21, an important part of the WASH complex associated with the retromer [86], [87].

1.4.4 Impaired mitochondrial dynamics and function

Mitochondrial dysfunction is a pervasive factor of both familial and idiopathic PD [88] . In PD models, genetic mutations adversely affect numerous mitochondrial processes. These include impaired mitophagy, mutations in mitochondrial DNA, disruptions in fission and fusion process, diminished mitochondrial biogenesis, and disturbed calcium homeostasis [66][89], [90], [91]. Increased expression of various human VPS35 forms leads to the fragmentation of mitochondria and the loss of neurons in different cell types, such as rat cortical neurons, M17 cells, and human fibroblasts. This effect is also observed in vivo, specifically in mouse substantia nigra neurons. Notably, the D620N variant induces more pronounced effects compared to the wild-type (WT) or R524W VPS35 variants [92] . Inhibiting the mitochondrial fission protein DLP1 with mdivi-1 can counteract the deleterious effects induced by VPS35 mutations. This suggests that this mutations can disrupt the mitochondrial functioning and dynamics and thus causes neurodegeneration [66]. Moreover, these mutations provoke mitochondrial dysfunction across various cellular models,

characterized by increased ROS production, impaired bioenergetic function, decreased ATP levels and diminished mitochondrial membrane potential [93]. The D620N mutation in VPS35 strengthens its interaction with DLP1, leading to the improper recycling of DLP1This increased degradation of mitochondrial DLP1 complexes is probably facilitated by their transportation to lysosomes for breakdown through the mitochondrial-derived vesicles (MDV) pathway. As a result, this mechanism probably leads to the fragmentation of mitochondria and the loss of neurons [94], [95]. Neurons deficient in VPS35, observed both in cultured cells and live animals, show high level of mitochondrial fragmentation, suggesting impaired mitochondrial fusion. This defect can be corrected by introducing wild-type, but not the D620N VPS35 form, suggesting that VPS35 mutations hinder MFN2-dependent mitochondrial fusion [96]. The decrease in MFN2 levels was caused by an increase in its ubiquitination and subsequent degradation by the proteasome, which was driven by elevated levels of MUL1.

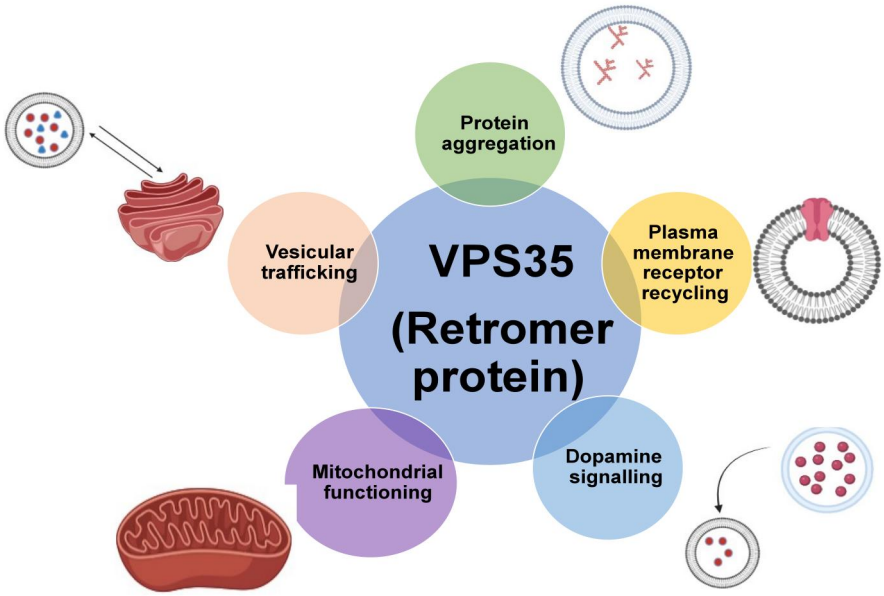

Fig 1.2. Role of the Vps35 protein in cellular physiology.

1.5. Physiological effects of Vps35 mutation

VPS35 deficiency is linked to neuropathological characteristics relevant to Parkinson's disease (PD). Deleting one copy of the VPS35 gene cause deficits, such as the α-synuclein build-up, decreased dopamine (DA) levels, reduced tyrosine hydroxylase (TH) protein, and fewer DA neurons in both the SNpc and striatum (STR), along with impaired locomotor behaviour [97] . Additionally, we observed altered lysosome morphology in VPS35-deficient DA neurons, characterized by

enlarged lysosome-associated membrane glycoprotein 1 (Lamp1+) vesicles and a decrease in Lamp2a+ vesicles. This reduction in Lamp2a causes increase in α-synuclein level in protein deficient neurons. Introducing Lamp2a can reduces the α-synuclein levels, indicating that it is crucial for degradation via chaperone-mediated autophagy (CMA). Interestingly, VPS35 or retromer deficiency, as well as the D620N mutation, impaired the retrieval of Lamp2a from endosomes to the Golgi, leading to its accelerated lysosomal degradation. These findings suggest that VPS35 is essential for regulating Lamp2a in DA neurons, potentially contributing to PD pathology in the context of VPS35 deficiency [98].

1.6. Phytochemicals

Medicinal plants can show their impact on metabolic processes because they are rich in nutritional values and give significant health benefits [99]. In PD research, during the past decades bioactive compounds of the medicinal plants have become very valuable drug for treatment. Although numerous drugs, such as L-dopa, MAO-B inhibitors, and dopamine agonists, are available to treat PD, they primarily address dopamine loss and cannot fully alleviate symptoms or stop disease progression. Current PD medications do not offer the long-term therapeutic benefits patients desire. The scientist are now focusing on the compounds that can show anti-parkinsonian activity and can have natural origin as a therapeutic target [100], [101]. Studies on medicinal plants and their phytochemicals can reveal that they have a healing properties along with lesser side effects because they can show anti-inflammatory and anti-oxidants properties. All these properties of bioactive compounds can demonstrate their antiparkinsonian activity [102], [103].

They can treat PD by several mechanism. These mechanisms include lowering α-synuclein aggregation, reduces level of caspase 3,8 and 9 by apoptosis inhibition and prevent dopaminergic neurons death [99]. The other mechanisms of action of these phytochemicals are to lowers the expression of proinflammatory cytokines to address the deletion of dopamine [99] [102], [104], [105]. In addition to the reductionist approach of deriving drugs from natural source it is imperative to pinpoint and elucidate the bioactive components responsible for these therapeutic effects. Phytochemicals, which are non-nutritive secondary metabolites, play a pivotal role in the realm of drug discovery and development, continuing to serve as a rich source of novel pharmaceuticals [106], [107]. Medicinal plants are abundant in various phytochemicals, encompassing a diverse array of secondary metabolites, including polyphenols (such as phenolic acids, anthocyanins, proanthocyanidins, flavonols, and tannins), alkaloids , fatty acids and isoprenoids (like sesquiterpenes, diterpenes, triterpenes, steroids, and saponins) [108]. These diverse constituents interact with various enzymes and cell receptors. Despite the longstanding tradition of employing various medicinal plants across diverse cultures to treat cognitive disorders, only a selected few have undergone rigorous scientific investigation to substantiate the pharmacological foundations of their therapeutic effects [109], [110], [111].

1.6.1 Effects of Phytochemicals on α-synuclein aggregation

It is a presynaptic protein in the brain having 140 residue of amino acids and is essential for the trafficking and fusion of synaptic vesicles and plays a key role in regulating dopamine release at presynaptic terminals [112], [113] . In a healthy human brain, α-synuclein is present at a physiological concentration of 1 μM, and in cerebrospinal fluid, it is 70 pM. α-synuclein in its native form is an unfolded monomer but when it binds with lipid vesicles it forms an alpha- helical structure. When destabilized, α-synuclein misfolds and aggregates within neurons [114] . Targeting the aggregation, oligomerization, fibrillation, and propagation of α-synuclein to reduce its toxicity has become a key therapeutic objective for slowing or halting the progression of the disease. Recent studies on phytochemicals and plant extracts can highlight that they can have a potential to show neuroprotection against PD due to their anti-oxidants and anti-inflammatory effects [115], [116], [117] . In dopaminergic neurons, the formation of intracytoplasmic inclusions containing α-synuclein, synphilin-1, and ubiquitin gives rise to Lewy bodies, a distinctive pathology of Parkinson's disease (PD) [118], [119] . The process of α-synuclein aggregation initiates with dimer formation, followed by the formation of small oligomers/protofibrils. These protofibrils can form beta-sheet-rich α-synuclein fibrils. As a result, they develop into aggregated α-synuclein, that are main constituents of Lewy bodies [120] . Hence, the oligomerization of alpha-synuclein monomers is the crucial initial stage in the multistep process of alpha-synuclein-induced neuronal toxicity, ultimately resulting in the formation of intracytoplasmic inclusions and fibrils [118], [121] . The inhibition of oligomerization and fibrils by the phytochemicals can become a key therapeutics target in PD. These plant extracts can target pathogenic stages of α -synuclein and shows neuroprotective effects [122], [123], [124] . The α -synuclein degradation can be affected by vps35 gene mutation, this mutation can affect the lysosomal degradation of alpha synuclein by reducing the levels of Lamp2a receptor that can be used in lysosomal degradation [78] . But it's not always that alpha synuclein can be accumulated or build up in body due to gene mutation, sometimes the aggregates of alpha synuclein can be formed prior to the disease onset by several other means like there is a connection between the gut and brain. This is a reciprocal connection in the gut and brain by a vagus nerve, (A tenth cranial nerve) that can transport the important metabolites from gut to brain [125], [126] . By extensive research and novel findings suggest that there is a link of gut in the neurodegeneration and the aggregation of alpha synuclein. In gut due to the dysbiosis i.e. change in the microbial population of the gut the certain microbes changes can lead to production of certain metabolites that can lead to alpha synuclein buildup in the gut and these protein aggregates can be transported to the brain via vagus nerve. These alpha synuclein in brain can lead to loss of dopamine producing neurons and affect dopamine signalling and causes the disease [127], [128] .

Research indicates that the gut microbiota influences both CNS and ENS functions, highlighting the essential role of metabolites and cellular components originating

from the gut. These factors from the gut significantly contribute to maintaining brain balance [126] . Specifically, the metabolites and neurotransmitters produced by gut microbiota aid in communicating with the CNS/ENS, affecting neuromodulation. Importantly, these compounds from the gut microbiota help in communication with the CNS and ENS, influencing neuromodulation. Many of these substances regulate crucial processes like neurogenesis, blood-brain barrier integrity, myelination, synaptic pruning, and glial cell function[128], [129] .

Studies suggest that misfolded proteins associated with neurodegenerative diseases (NDDs) can travel from the gut to the brain. In Parkinson's disease (PD), for instance, misfolded α-synuclein aggregates have been detected in the ENS. An imbalance in gut microbiota, known as dysbiosis, worsens this process by aiding in the buildup and transfer of misfolded proteins. This underscores the complex connection between gut health, protein aggregation, and neurodegeneration [128].

Phytochemicals possess antioxidant, anti-inflammatory, antiapoptotic, and neuroprotective properties that aid in therapy. Targeting phytochemicals that stabilize, enhance clearance, degrade misfolded proteins, solubilize oligomers, or inhibit the spread of alpha-synuclein aggregates is a pharmacologically viable approach and represents a clinically significant therapeutic strategy for Parkinson's disease [130].

Curcumin, also known as diferuloylmethane, is a prominent natural compound derived from turmeric and is extensively studied for drug discovery and development [131] . Its antioxidant, anti-inflammatory, and antiapoptotic properties make it beneficial in the treatment of neurodegenerative disorders, including Parkinson's disease [132], [133], [134] . Studies have found that curcumin possesses anti-fibrillogenic properties, as it inhibits the formation of alpha-synuclein fibrils and destabilizes already formed fibrils [135]. It strongly attaches to the hydrophobic non-amyloidogenic part of alpha-synuclein [136].

This could be a very novel therapeutics approach by targeting alpha-synuclein in PD, because by targeting this alpha-synuclein aggregates these phytochemicals can help in combating the disease, hence there is connection of alpha-synuclein aggregates formation prior to the disease onset and have involvement of GI tract also. In this approach we don’t have to search for the drugs that are BBB positive because here the hunt for drugs is based upon the approach of GI absorption that the agents have to be solubilised in GI tract for their action [137].

CHAPTER-2

METHODOLOGY

2.1. Sources

Database used: PubMed, PubChem, IMPPAT 2.0, Protein Data Bank (PDB), UniProt.
Software used: Open Babel, PyRx, CB-Dock2, Biovia Discovery Studio Visualizer, PyMOL, CarcinoPred-EL, PkCSM.

2.2. Workflow

2.2.1 Phytochemicals which show BBB permeability

From a comprehensive literature review, VPS35 (D620N) comes out to be as a potent target against PD. To identify ligand molecules, plants with various therapeutic applications like Anti-Parkinson, Anti- Cancer, Anti- Inflammatory were selected. The structures of plant-specific phytochemicals were retrieved from IMPPAT 2.0 and then filtered based on their ability to permeate the BBB and further can be used for investigation. The sequential workflow process is illustrated in the figure 3.1.

2.2.2 Phytochemicals which Target α-synuclein aggregation

A literature review identified Vps35 (D620N) as a target molecule for repurposing an antagonist to address abnormal trafficking of protein that is associated with α-synuclein aggregation, a significant cause of Parkinson's disease. Phytochemicals known to target α-synuclein aggregation were compiled from the literature, and their chemical structures were retrieved from PubChem. Among these, those with high gastrointestinal (GI) absorption were selected as ligands for further investigation. The protocol flowchart is shown in Figure 3.2.

IMPPAT 2.0

IMPPAT 2.0 (Indian Medicinal Plants, Phytochemistry, And Therapeutics 2.0) is the largest manually curated database of phytochemicals. It was created by digitizing available data on traditional Indian medicinal plants, capturing associations between plant parts, phytochemicals, and their therapeutic uses. This integrated platform

emphasizes the wealth of information within traditional Indian medicine and promotes natural product-based drug discovery.

2.3. Data Extraction

A literature review identified 14 Indian medicinal plants. The medicinal plants selected can have properties like anticancer, , antimalarial, anti-allergic, anti-diabetic, analgesic, antimicrobial, cytotoxic, antioxidant, hepatoprotective, vasorelaxant, neuroprotective, anti-inflammatory antibacterial, antiproliferative, antifungal, antiulcer, antidiarrheal, immunomodulatory, antipyretic, antiplasmodic, antihistaminic, anthelmintic, astringent, antihyperglycemic, antispasmodic and others targeting α-synuclein aggregation were mentioned in the table. The 3D structure of these phytochemical compounds specific for each plant were retrieved from IMPAAT2.0 by individual entry. Additionally, the structure of Curcumin , a Reference drug that is used in Parkinson disease, was also downloaded from PubChem and used as a control drug. The ChEMBL database was used to evaluate various properties of the ligands, such as molecular weight. From the initial set of 495 drugs, a subset was chosen for further molecular docking studies. For FDA approved drugs , the drug libraray can be downloaded from DrugBank, using Amantadine as a the Reference drug and its structure is downloaded from PuChem. The target protein Vps35 is downloaded from Protein Data Bank(PDB) in .pdb format and further D620N mutation can be introduced.

Table 2.1 List of Medicinal plants with their therapeutics properties

S.No.	Name of medicinal plant	Family	Number of phytochemical entries	Source of phytochemicals	Activity	References
1.	Albizia lebbeck	Fabaceae	108	Bark, flower, fruit, leaf, root, seed, wood	Anticancer, anti-nociceptive, anti-inflammatory, antimalarial, antiallergic, neuroprotective	[138], [139], [140]
2.	Asparagus officinalis	Asparagaceae	51	flower, leaf, root, seed, shoot	Anti-diabetic, anti-cancer, anti-fungal, antimicrobial	[141], [142], [143]
3.	Asparagus racemosus	Liliaceae	40	Bark, flower, fruit, leaf, root, wood,	Antiulcer, antioxidant, and antidiarrhoeal, antidiabetic and immunomodulatory, antitumor	[144], [145]
4.	Bauhinia	Fabaceae	20	Bark, root, seed,	Analgesic,	[146],

	racemosa			stem, wood	antipyretic, anti–inflammatory, anti-plasmodic, antimicrobial, antihistaminic	[147], [148]
5.	Butea monosperma	Fabaceae	77	Bark, flower, plant exudate, root, seed, whole plant	Anti-tumor, anti-microbial, anti-helmintic, anti-inflammatory, astringent	[149], [150]
6.	Cedrus deodara	Pinaceae	189	Bark, flower, leaf, plant exudate, root, seed, wood, whole plant	Anti-inflammatory, analgesic, anti-hyperglycaemic, anti-spasmodic, antibacterial, insecticidal, anti-apoptotic, immunomodulatory, anti-malarial, anti-fungal, anti-ulcer, anticonvulsant, anti-cancer	[151], [152]
7.	Croton tiglium	Euphorbiaceae	33	Seed	Anti-bacterial, anti-fungal, analgesic, anti-inflammatory, anti-HIV, anti-tumor	[153], [154], [155]
8.	Datura metel	Solanaceae	104	Aerial, part, bark, flower, fruit, leaf, root, seed, stem, whole plant	Anti-proliferative, anti-inflammatory, antioxidant, antipyretic, and analgesic	[156], [157], [158]
9.	Euphorbia hirta	Euphorbiaceae	129	Aerial, part, bark, flower, leaf, plant exudate, root, stem, whole plant	Immunomodulatory, anti-inflammatory, analgesic, anti-tumor	[159]
10.	Moringa oliefera	Moringaceae	200	Bark, flower, fruit, leaf, root, seed, stem, whole plant	Antioxidant, anti-cancer, anti-inflammatory	[160]
11.	Plantago major	Plantaginaceae	46	Aerial part, flower, leaf, root, seed, whole plant	Hepatoprotective, Anti-hypercholesteremia, Anti-atherosclerosis,	[161], [162], [163]

					anti-inflammatory, analgesic, antifungal, antiviral, anti-bacterial, anti-cancer	
12.	Taxus wallichiana	Taxaceae	181	Bark, fruit, leaf, Root, stem, wood	Analgesic, anti-inflammatory, immunomodulatory, antispasmodic antiallergic, anticonvulsant, antiosteoporotic,anticocicepti ve, antimicrobial, antiplatelet, antipyretic	[164], [165], [166]
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	[167], [168]
14.	Vitex negundo	Verbenaceae	228	Bark, flower, fruit, leaf, root, seed, stem	Antihelmintic, anti-inflammatory, anti-proliferative, antioxidant	[169]

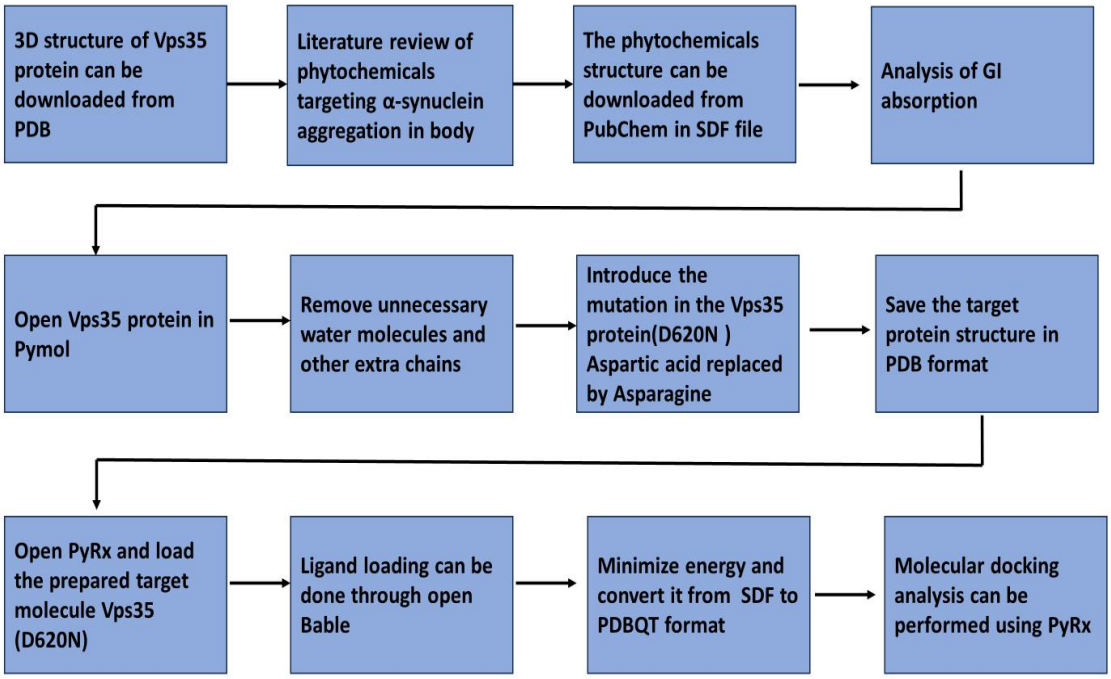

Fig. 2.1 Process of the steps involved in Molecular Docking of Phytochemicals targeting α-synuclein aggregation.

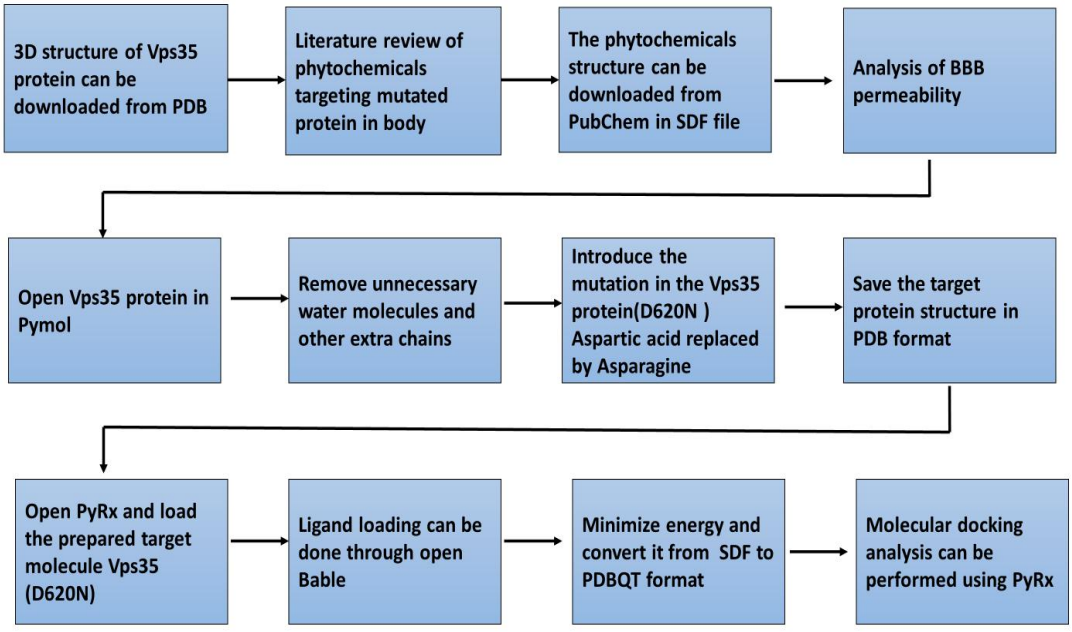

Fig. 2.2 Process of the steps involved in Molecular Docking of Phytochemicals from Medicinal Plants

2.4. BBB Permeability Analysis

Out of the 1580 phytochemicals, 390 were found to be capable of passing through BBB. This analysis was conducted using an online tool for predicting BBB permeability by SWISS ADME. The Swiss ADME tool can assess whether the selected ligands are BBB+ or BBB-, indicating their potential to cross the blood-brain barrier. This freely accessible web tool predicts the pharmacokinetic, physicochemical, absorption, distribution, metabolism, and elimination properties of molecules. To analyze the properties of ligands using Swiss ADME, the canonical SMILES for the ligands can be obtained from PubChem. For the other 105 phytochemicals compounds that did not show BBB permeability, gastrointestinal (GI) absorption can be considered as a factor for targeting alpha-synuclein aggregation. For the FDA- approved drugs, from the 3,674 drugs can be subjected for molecular docking, out of that only 11 compounds exhibited blood brain permeability.

2.5. Molecular Docking

Subsequently, the interaction between the receptor and ligand was analyzed using molecular docking with the PyRx web server, followed by additional procedures.

2.5.1 Preparation of the Target Receptors

The protein structure with PDB ID 5OSI, which has a resolution of 2.52 Å, was retrieved from the database. PyMOL was utilized to open the .pdb file, check for errors, and perform necessary corrections. These corrections included removing unnecessary ligands such as other bound protein complexes and inhibitors attached to the protein, as well as extra chains, hydrogen atoms, and all heteroatoms. Water molecules were also removed from the protein chain to obtain a refined protein molecule. Mutations were introduced using PyMOL's wizard feature, specifically replacing aspartic acid at residue 620 with asparagine, thus creating the D620N missense mutation in the protein. The mutated protein was then saved in .pdb format. The mutated protein can be converted into macromolecules using PYMOL.

2.5.2 Preparation of the Ligand Molecules

In the search for a promising inhibitory drug for Vps35, a collection of 1580 phytochemical ligands was used for docking experiments. These ligands were converted into Pdbqt format in Open Babel within PyRx. After undergoing energy minimization, the ligands, now can be converted from .sdf to .pdbqt format, were ready for further analysis. In case of FDA- approved drugs, after downloading the structure, the downloaded drugs can be converted into .sdf format for docking through Open Babel.

2.5.3 Molecular Docking Studies

PyRx functions as a virtual screening tool for evaluating compound libraries, using a variety of well-known open-source applications. It is an open-source software, that is used for the virtual screening of libraries to identify potential drug targets. It includes a comprehensive suite of various software tools, making it a valuable resource for computer-aided drug discovery. In this study, Open Babel within PyRx was utilized for importing ligands and performing energy minimization, while AutoDock Vina was employed for docking simulations. After preparation, both the protein and ligand were displayed in the AutoDock Tab. Molecular docking experiments were conducted using the AutoDock Vina program integrated into the PyRx platform. Blind docking procedures were executed using the Vina methods embedded within PyRx. After the completion of docking process, the results were saved as .csv and output files.

2.5.4 Docking Analysis

To identify efficient ligands, .csv files for each medicinal plant were analyzed, and potential natural inhibitors were selected based on their docking scores. For FDA-approved drugs, the drug showing the highest affinity for the target protein was chosen. Subsequently, ligands that could pass BBB were selected for further studies and analysis.

2.5.5 Docking Result Validations

To validate the results, CB-DOCK2 was used. CB-DOCK2 is an advanced blind docking server designed for virtual screening. The validation results for both the phytocompounds and the FDA-approved drugs are presented in the table 3.4 & 3.6.

2.5.6 Analyzing Protein-Ligand Interactions

The interactions were examined using Discovery Studio Visualizer and PyMOL. Compounds with a Root-Mean-Square Deviation (RMSD) below 1 Å and binding free energy greater than -8.00 kcal/mol were prioritized and further aasessed for blood-brain barrier. From the initial 1580 phytochemical compounds tested, only 390 showed BBB permeability. However, only 7 compounds with binding energy exceeding -8.00 kcal/mol were selected. Additionally, out of 105 molecules targeting alpha-synuclein, only 9 were chosen. The chemical structures of these BBB-permeable drugs were obtained from PubChem in Structure Data Format (SDF). The output files of the selected ligands were analyzed using Discovery studio and Pymol for its 2D and 3D interactions with the protein molecules. These platforms enable the visualization of ligand binding sites on the perotein, detailing the number of interacting residues, as well as identifying the specific amino acids involved in these interactions.

2.5.7 Swiss ADME-based ADME assessments for the ligand

The ADMET properties were predicted using Swiss ADME, [170]. ADME analysis was conducted on seven selected physicochemical compounds utilizing the Swiss ADME framework. This computational tool enabled an in-depth examination of pharmacokinetic properties. The study evaluated essential factors, including pharmacokinetics, gastrointestinal absorption probability, blood-brain barrier permeability, P-glycoprotein substrate status, adherence to Lipinski's Rule, potential violations of this rule, aqueous solubility, lipophilicity, and bioavailability. Lipinski's Rule comprises parameters that are used to check the drug-likeliness of a molecule. These criteria serve as benchmarks for determining the likelihood of a compound possessing optimal pharmacokinetic properties for effective oral delivery. The toxicity and carcinogenic properties of the selected compounds can be tested by PkCSM and CarcinoPred-EL.

2.5.8 Toxicity and Carcinogenicity Analysis

CarcinoPred-EL, a web-based tool utilizing ensemble learning methods, was employed to predict the carcinogenic potential of these compounds. PkCSM, a toxicity prediction tool that relies on graph-based signatures measures the extent of toxicity [171]. The various properties of the hit compounds were analyzed just by input of SMILES extracted from IMPPAT2.0 in each tool.

CHAPTER-3

RESULT & DISCUSSION

3.1. Docking result

Phytochemicals from 14 Indian medicinal plants with blood-brain barrier permeability were subjected to molecular docking, alongside the reference drug Curcumin. Among all the phytochemicals docked from the 14 different plants, only those from 4 plants showed efficient inhibition against the protein, as shown in the table. Reference drug shows a docking score of -6.3 kcal/mol with Vps35(D620N). The phytochemical with the most negative binding energy score, indicating the most stable ligand-protein interaction complex, was derived from the Datura metel plant with an IMPAAT ID of IMPHY002029, emerging as the most potent inhibitor. The docking score of selected compounds is -9.0 kcal/mol and 0.0 Å RMSD value. For validation and further analysis, the top 7 compounds with the highest binding scores were selected. Among the FDA-approved drugs, Irinotecan had the most negative binding energy score of -9.6 kcal/mol, while the reference compound had a score of -4.8 kcal/mol. A threshold of >-8.5 kcal/mol was set to screen drugs based on their binding energy scores. Compounds that met this threshold were then analyzed for BBB permeability using Swiss ADME. The top three FDA- approved drugs chosen were Irinotecan, Tirilazad and Alectinib and their binding affinity scores are -9.6, -8.6 and -9.1 kcal/mol respectively can be selected for further investigation.

Table 3.1 Docking score of the Phytochemicals obtained from Medicinal Plants

Compound	Docking scores	IMPPAT Phytochemical Identifier	Medicinal plant	Solubility Class (SILICOS-IT)	BBB Permeant	PGP substrate
Luteoxanthin	-9	IMPHY002029	Utrica diocia	Moderately soluble	Yes	Yes
Isowithametelin	-8.7	IMPHY010687	Datura metel	Moderately soluble	Yes	Yes
withametelin (daturilin)	-8.5	IMPHY003277	Datura metel	Moderately soluble	Yes	Yes
Withametelin B	-8.2	IMPHY009120	Datura metel	Moderately soluble	Yes	Yes
Diosgenin	-8.1	IMPHY003681	Asaparagus racemosus	Moderately soluble	Yes	No
Daturilinol	-8	IMPHY008964	Datura metel	Moderately soluble	Yes	Yes
Sarsasapogenin	-8	IMPHY012274	Asparagus officinalis	Moderately soluble	Yes	No

Table 3.2 Drug likeness, pharmacokinetics and docking score of the phytochemicals targeting α-synuclein

Compounds	Docking scores	GI absorption	Solubility class	BBB permeant	Pgp Substrate	CYP 1A2 inhibitor	CYP2 C19 inhibitor	CYP 2C9 inhibitor	CYP2 D6 inhibitor	CYP3A4 inhibitor
withanolide A	-8.4	high	Moderately soluble	No	Yes	No	No	No	No	No
Rottlerin	-8.6	low	Poorly soluble	No	No	No	Yes	No	No	No
Isohopeaphenol	-8.8	Low	Insoluble	No	Yes	No	No	No	No	No
hypericin	-8.7	low	Poorly soluble	No	No	No	Yes	Yes	No	No
Hinokiflavone	-8.6	low	Poorly soluble	No	No	No	No	Yes	No	No
Hederagenin	-7.8	High	Moderately soluble	No	No	Yes	No	No	No	No
beta-Amyrin	-8.3	low	Poorly soluble	No	No	No	No	No	No	No
Astaxanthin	-8	Low	Moderately soluble	No	Yes	No	No	No	No	No

3.2. Protein- Ligand Interactions

After completing the docking process, the output files of the top hit phytochemicals can be used to visualize the interacting residues between the ligands and the target proteins. These interactions can be visualized using Biovia Discovery Studio Visualizer. The 2D and 3D interactions can be illustrated in the diagrams.

Table 3.3 2D interactions of both the top hit Phytochemicals and FDA- approved drugs

Sr.no.	Compounds	Interacting residue
1.	IMPHY002029	Leu583, Leu579, Pro530, Arg526, Phe527, Gln488, Pro531, Thr528, Pro531, Phe534, Tyr537, Gln538, Phe541, Glu593, Glu545, Lys544
2.	IMPHY010687	Gln488, Phe527, Arg526, Leu579, Pro531, Phe534, Pro530, Val491, Gly492, Ile495, Arg499
3.	IMPHY003277	Gln538, Phe534, Tyr537, Ala590, Gln586, Leu589, Glu593, Phe541
4.	IMPHY009120	Gln586, Arg499, Leu589, Ala590, Phe541, Tyr537, Phe534, Gln538, Ile495, Pro531, Gly492,
5.	IMPHY003681	Arg637, Leu589, Gln586, Glu593, Gln538, Arg542, Glu545, Phe541
6.	IMPHY008964	Lys639, Glu593, Gln586, Leu589, Arg637, Phe541, Gln538, Phe534, Tyr537, Ala590,
7.	IMPHY012274	Leu579, Phe527, Arg526, Pro530, Gln488, Pro531, Gly492, His496, Arg499, Ile495, Phe534
8.	Curcumin (Reference)	Glu501, Ile509, Leu498, Thr512, His516, Ala513, Phe494, Phe517, Leu490, Arg493, Leu497

Sr. no.	Compounds	Interacting Residues
1.	Tirilazad	Pro530, Pro531, Ser489, Gln488, Gly492, Ile495, Arg499, Phe534, Gln538, Tyr537, Gln586, Ala590, Leu589.
2.	Irinotecan	Phe534, Gln538, Tyr537, Leu589, Ala590, Ile495, Ala535, Arg499, Gly492, Phe527, Gln488, Pro530, Pro531, Glu593
3.	Alectinib	Leu579, Phe534, Pro530, Leu583, Pro531 Ile495, Arg499, His496, Arg526, Phe527, Gln488, Gly492
4.	Amantadine (Reference drug)	Gln586, Phe541, Phe534, Gln538, Ala590, Tyr537

3.3. Validation of Result

After validating the top seven phytochemicals from Indian medicinal plants and the top three FDA-approved drugs using CB-DOCK2, the docking values were obtained. The docking scores from CB-DOCK2 differed from those obtained using PyRx, with a difference of approximately ≤ 0.5 kcal/mol between the two sets of scores. The docking scores of the compounds are presented in Table 3.4 and 3.5.

Table 3.4 Phytochemicals docking scores of the top seven Phytochemicals along with the reference compound from PyRx and CB-DOCK2

Target Protein	IMPPAT ID	PyRx	CB Dock 2
Vps35	IMPHY002029	-9	-8.8
	IMPHY010687	-8.7	-8.4
	IMPHY003277	-8.5	-8.0
	IMPHY009120	-8.2	-8.3
	IMPHY003681	-8.1	-7.8
	IMPHY008964	-8	-8.4
	IMPHY012274	-8	-8.1
	Curcumin(Reference)	-6.3	-6.8

Table 3.5 Docking scores of FDA- approved drugs of top three compounds from PyRx and CB-DOCK2

Target Protein	FDA approved drugs	PyRx	CB-DOCK
Vps35	Irinotecan	-9.6	-9.9
	Tirilazad	-8.6	-9.6
	Alectinib	-9.1	-8.5
	Amantadine (Reference)	-4.8	-4.5

3.4. ADME Analysis Results

The top hit compounds, both phytochemicals and FDA-approved drugs, were evaluated for ADME using the freely accessible tool. The resulting data, which includes the physicochemical properties, drug-likeness, and pharmacokinetics of the selected compounds, is illustrated in the table. The BOILED-EGG images and

bioavailability radar diagrams of the phytochemicals are recorded and presented in the figure.

Table 3.6 Physiochemical properties of the hit Phytochemical Compounds

Properties	Drug name			
	Luteoxanthin	**Isowithametelin**	**Withametelin**	**Withametelin B**
Molecular weight (g/mol)	600.88	436.58	436.58	452.58
Hydrogen-bond donors	2	0	0	1
Hydrogen-bond acceptors	4	4	4	5
Molar refractivity	185.76	124.69	124.69	125.85
Topological polar surface area (Å2)	62.22	52.60	52.60	72.83
Lipinski's rule of five	No, 2 violations	Yes,1 violation	Yes, 1 violations	Yes, 0 violations
Bioavailability Score	0.17	0.55	0.55	0.55
Log P [SILICOS-IT]	9.77	4.91	4.91	4.01
Solubility	-5.38	-5.09	-5.09	-4.27

Table 3.7 Physiochemical properties of the hit Phytochemical compound 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 3.8 Physiochemical properties of the hit FDA- approved drugs

Compound	Molecular weight (g/mol)	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Topological Polar Surface Area (Å2)	Solubility	Bioavailability score	Log P [SILICOS-IT]
Irinotecan	586.68	1	8	114.20	-7.28	0.55	4.33
Tirilazad	624.86	0	5	72.88	-6.25	0.55	4.07
Alectinib	482.62	1	4	72.36	-8.40	0.55	5.93

FDA drugs	GI absorption	Lipinski's rule of five	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
Irinotecan	High	Yes; 1 violation	No	No	Yes	Yes	Yes
Tirilazad	High	Yes; 1 violation	No	No	No	No	Yes
Alectinib	High	Yes; 0 violation	No	Yes	Yes	No	No

Table 3.9 Drug-likeness and pharmacokinetics of the hit Phytochemical Compounds

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

3.5. Toxicity and carcinogenicity Analysis:

It can predict the toxicity and carcinogenic properties of the hit compounds of both the Phytochemicals and FDA-approved drugs. The summary of results of the hit compounds for both Phytochemicals and FDA-approved drugs were presented in table.

Table 3.10 Toxicity and Carcinogenicity of the top hit Phytochemicals Compounds

BBB permeable Phytochemicals				
Compound	**Ames Toxicity**	**Oral Rat Acute Toxicity LD50 (mol/kg)**	**Max. Tolerated dose human (log mg/kg/day)**	**Carcinogenicity**
Luteoxanthin	No	2.192	-0.847	No
Isowithametelin	No	1.926	-0.446	No
Withametelin	No	1.926	-0.446	No
Withametelin B	No	2.193	-0.737	No
Diosgenin	No	1.855	-0.461	No
Daturilinol	No	2.024	-0.453	No
Sarsasapogenin	No	2.041	-0.388	No
α-synuclein Targeting Phytochemicals				
Withanolide A	No	2.987	-0.589	No
Hederagenin	No	2.856	0.139	No

Table 3.11 Toxicity and Carcinogenicity of the FDA- approved drugs

FDA drugs	**Ames Toxicity**	**Oral Rat Acute Toxicity LD50(mol/kg)**	**Max. Tolerated dose human (log/mg/kg/day)**	**Carcinogenicity**
Tirilazad	No	2.873	-0.599	No
Irinotecan	No	2.184	0.174	No
Alectinib	No	2.963	0.097	No

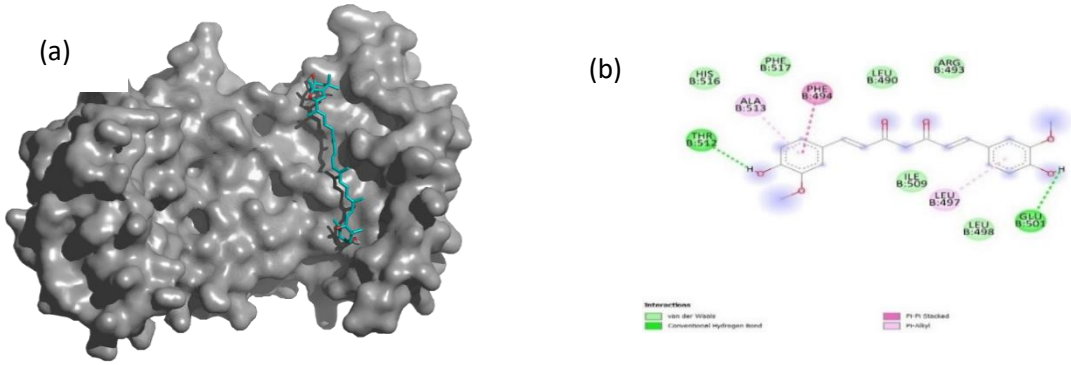

Fig. 3.1 (a) The three-dimensional representation depicting the binding mode of Luteoxanthin with Vps35 (D620N). (b) The two-dimensional image showing the binding pattern of the phytochemical, Luteoxanthin, with the Vps35 (D620N) protein.

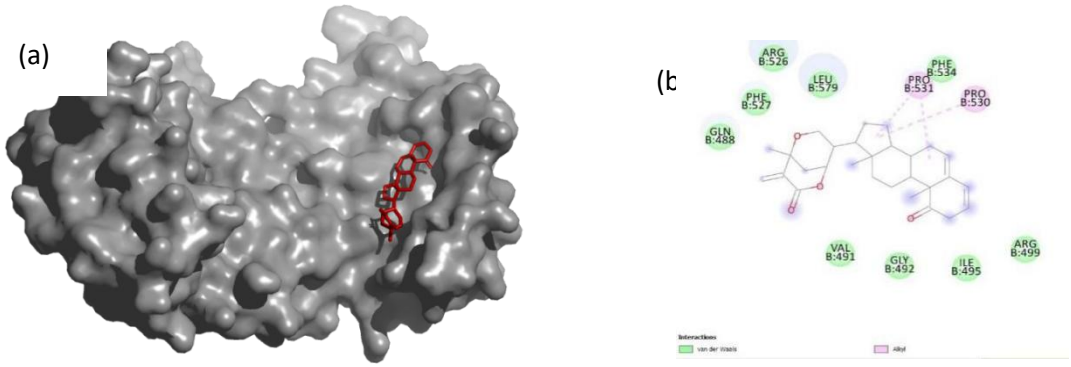

Fig. 3.2 (a) The three-dimensional representation depicting the binding mode of Isowithametelin with Vps35(D620N). (b) The two-dimensional image showing the binding pattern of the phytochemical, Isowithametelin, with the Vps35 (D620N) protein.

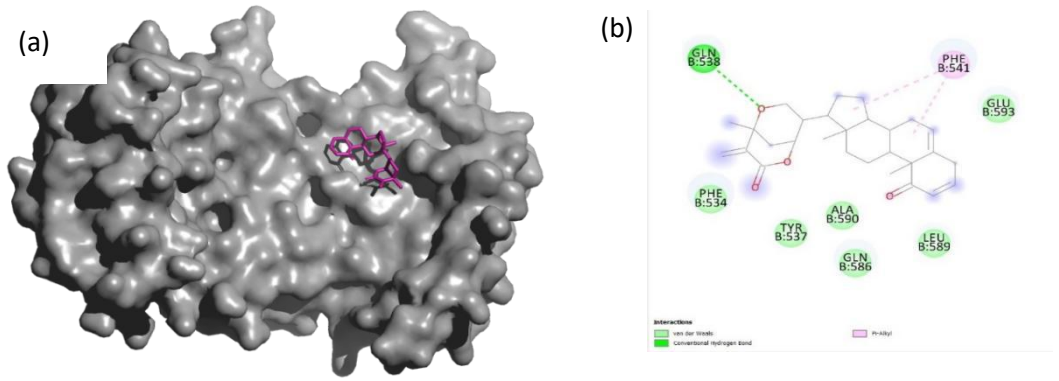

Fig. 3.3 (a) The three-dimensional representation depicting the binding mode of Withametelin with Vps35 (D620N). (b) The two-dimensional image showing the binding pattern of the phytochemical, Withametelin, with the Vps35 (D620N) protein.

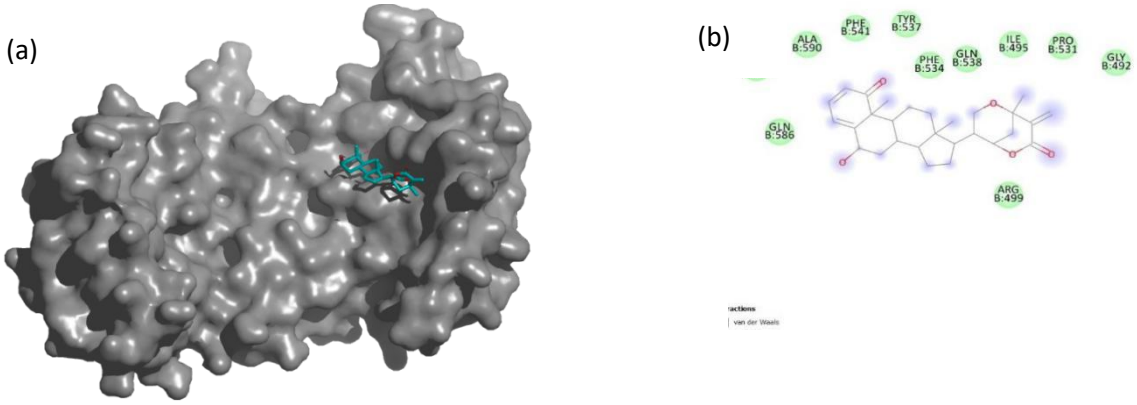

Fig. 3.4 (a) The three-dimensional representation depicting the binding mode of Withametelin B with Vps35 (D620N). (b) The two-dimensional image showing the binding pattern of the phytochemical, Withametelin B, with the Vps35 (D620N) protein.

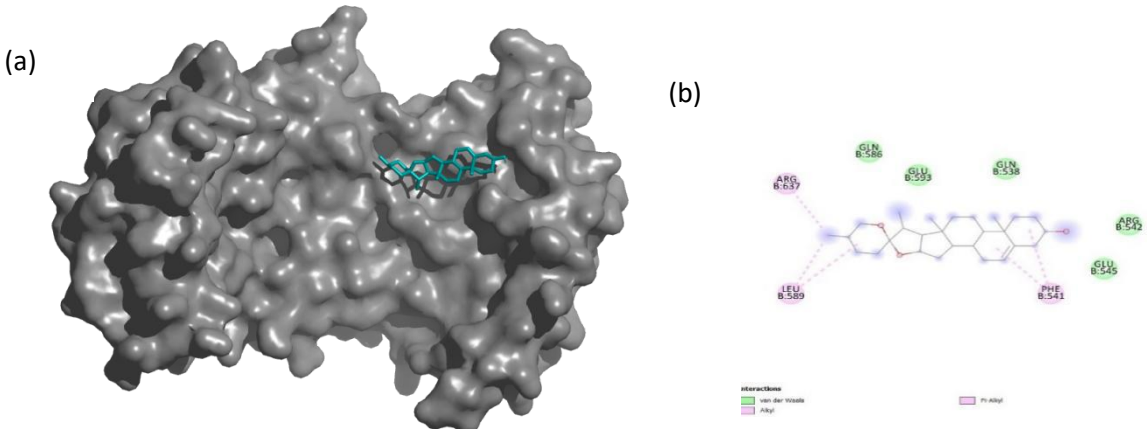

Fig. 3.5 (a) The three-dimensional representation depicting the binding mode of Diosgenin with Vps35 (D620N). (b) The two-dimensional image showing the binding pattern of the phytochemical, Diosgenin, with the Vps35 (D620N) protein

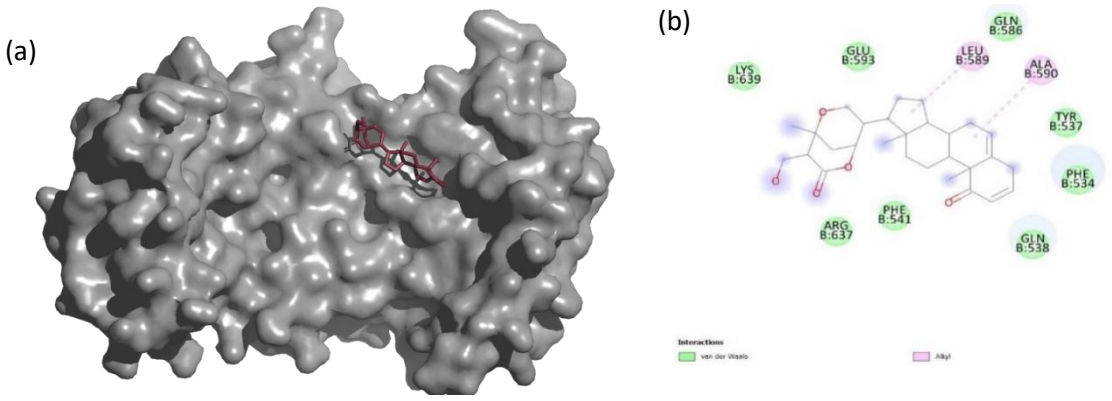

Fig. 3.6 (a) The three-dimensional representation depicting the binding mode of Daturilinol with Vps35 (D620N). (b) The two-dimensional image showing the binding pattern of the phytochemical, Daturilinol, with the Vps35 (D620N) protein

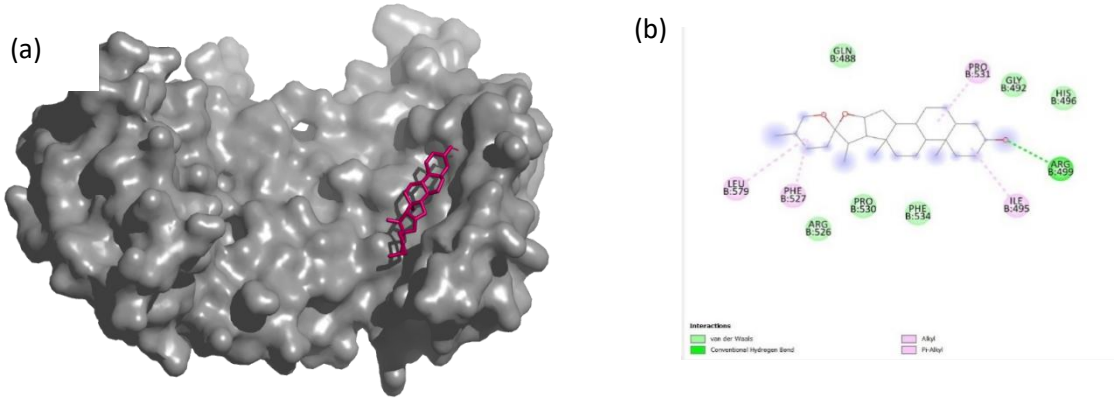

Fig. 3.7 (a) The three-dimensional representation depicting the binding mode of Sarsasapogenin with Vps35 (D620N). (b) The two-dimensional image showing the binding pattern of the phytochemical, Sarsasapogenin, with the Vps35 (D620N) protein.

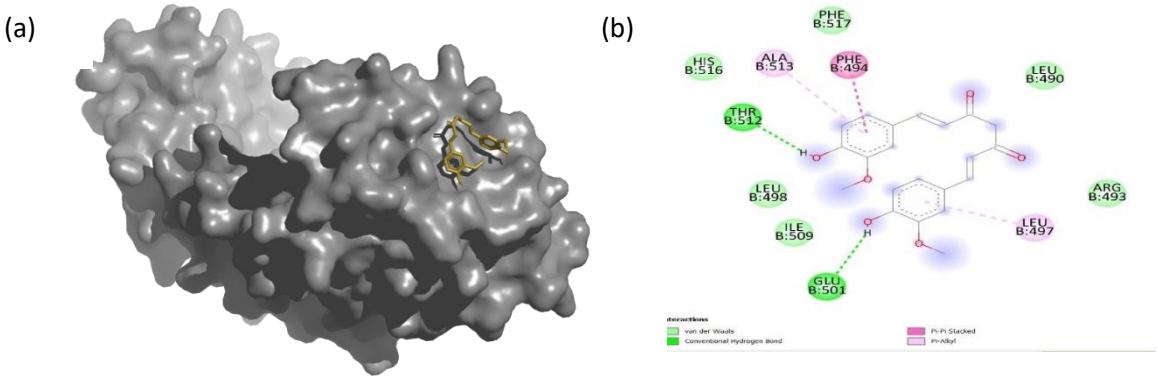

Fig. 3.8 (a) The three-dimensional representation depicting the binding mode of Curcumin (Reference) with Vps35 (D620N). (b) The two-dimensional image showing the binding pattern of the phytochemical, Curcumin (Reference), with the Vps35 (D620N) protein.

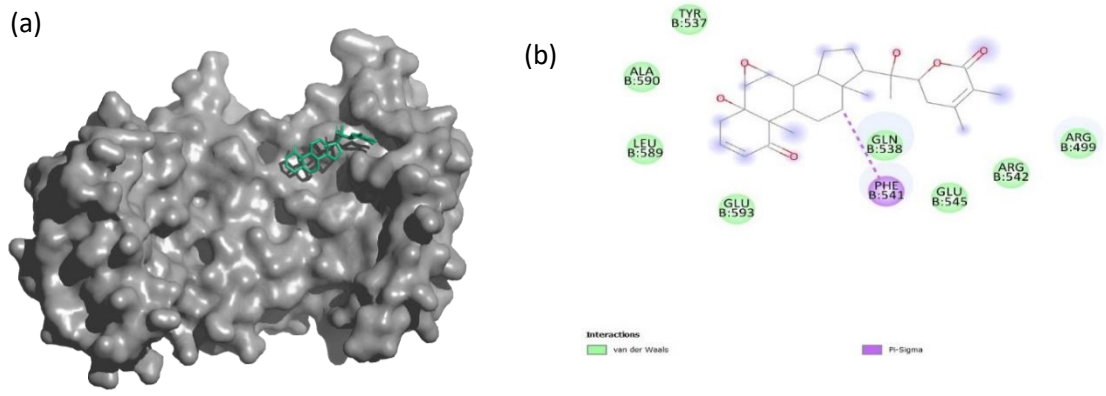

Fig. 3.9 (a) The three-dimensional representation depicting the binding mode of Withanolide A with Vps35 (D620N). (b) The two-dimensional image showing the binding pattern of the proposed phytochemical, Withanolide A, with the Vps35 (D620N) protein

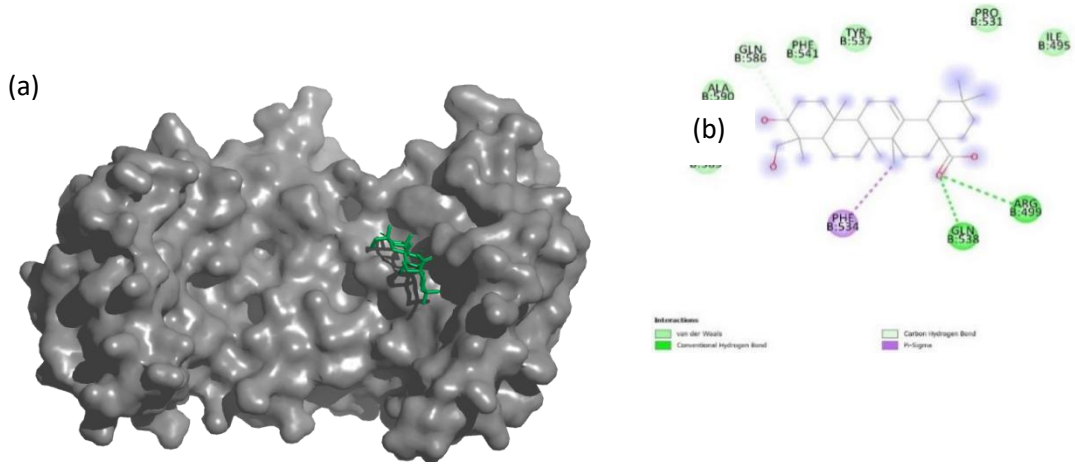

Fig. 3.10 (a) The three-dimensional representation depicting the binding mode of Hederagenin with Vps35 (D620N). (b) The two-dimensional image showing the binding pattern of the proposed phytochemical, Hederagenin, with the Vps35 (D620N) protein

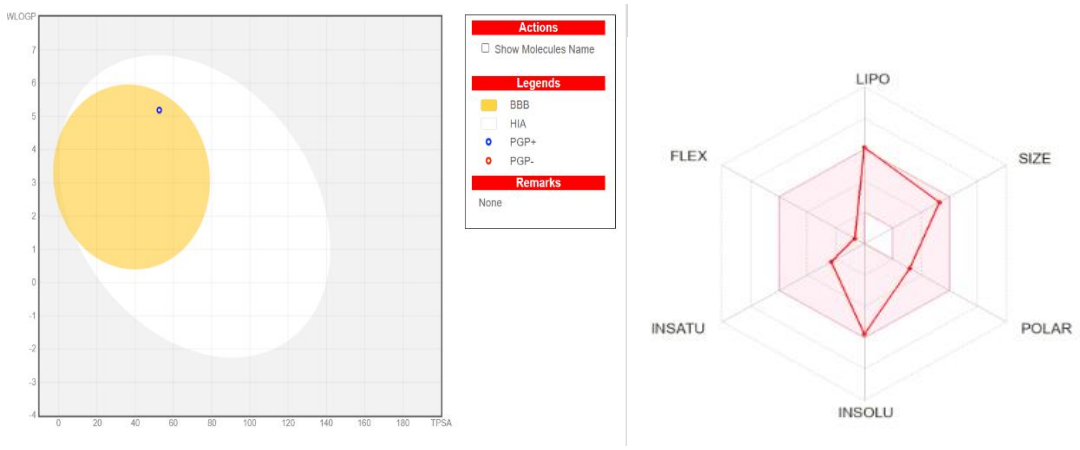

Fig. 3.11 BOILED EGG image of Isowithametelin derived by ADME analysis and bioavailability radar diagram of Isowithametelin showing a radar plot that is positioned entirely within the designated pink area

DISCUSSION

VPS35 is an essential part of Retromer, essential for recycling membrane proteins, and maintaining cellular homeostasis, autophagy, synaptic transmission, lysosomal degradation and protein distribution. In PD, mutations in Vps35, especially the D620N mutation, impair its function, leading to neuronal degeneration, alpha-synuclein aggregation, mitochondrial dysfunction, and disrupted autophagy. These dysfunctions result in altered neuronal signaling and impaired protein clearance, contributing to the pathogenesis of PD. Understanding these mechanisms positions Vps35 as a key therapeutic target in the fight against Parkinson's disease. Currently, there are limited medications or treatments available that can slow down the progression of the disease or prevent its onset. Only medication present is to halts the symptoms or slows down the progression of symptoms Currently, levodopa is the only medication available for PD patients, highlighting the urgent need to discover alternative drugs that offer therapeutic benefits without causing significant side effects and preferably have a natural origin. This study focuses on two findings: first one is the actions of plant-derived phytochemicals from certain medicinal plants that may possess anti-Parkinson properties and the other one is FDA approved drugs.

Curcumin, a well-known anti-cancer compound from the Curcuma longa plant, which also exhibits anti-parkinsonian properties, was used as a reference drug for identifying potential lead compounds. The aim is to select natural compounds as potent inhibitors against the Vps35 protein. In validation docking performed using PyRx, curcumin showed a binding energy score of -6.3 kcal/mol against the mutated Vps35 protein. Curcumin is a polyphenolic compound obtained from turmeric and it has strong potent therapeutical properties [172]. These qualities make it a promising therapeutic and nutraceutical option for treating Parkinson's disease[173].

Curcumin protects mitochondria and neurons from the harmful effects of ROS by donating a hydrogen ion. Curcumin has been demonstrated to inhibit the aggregation of alpha-synuclein oligomers. [174] . Curcumin, the active component in turmeric, possesses antioxidant, anti-apoptotic, and anti-inflammatory properties that safeguard tissues from the damaging effects of reactive oxygen species (ROS) [175]. A docking threshold of -8 kcal/mol or lower was chosen to identify ligands with potential inhibitory effects against the Vps35 protein.

Table 4.1 shows the phytochemicals that meet this binding affinity criterion and show high BBB. Among all the compounds, seven—Luteoxanthin (IMPHY002029), Isowithametelin (IMPHY010687), Withametelin (IMPHY003277), Withametelin B(IMPHY009120), Diosgenin (IMPHY003681), Daturilinol (IMPHY008964), and

Sarsasapogenin (IMPHY012274)—exhibited the most negative binding energy scores. Based on the Binding affinity three compounds Luteoxanthin, Isowithametelin and Withametelin (Daturilin) have the most negative binding energy score. Among the phytochemicals, Isowithametelin, Withametelin, and Daturilinol can be derived from the leaves of the Datura metel plant and belong to the class of steroids based on chemical classification. Isowithametelin has cytotoxic activity and cancer chemopreventive potential. Withametelin also possesses antifungal and neuroprotective activities, along with cytotoxic effects against cancer. Luteoxanthin (IMPHY002029), derived from the Urtica dioica plant, exhibits antioxidant, anti-inflammatory, and cardiovascular properties. Diosgenin, another steroid, is derived from the leaves of Asparagus racemosus and has antioxidant, antidiabetic, and immunomodulatory properties. Sarsasapogenin, derived from the Asparagus officinalis plant, shows antidiabetic, anticancer, antifungal, and antimicrobial properties. Further docking validation can be performed using CB-DOCK2 to confirm the effectiveness of these compounds as inhibitors of the target protein.

Table 3.2 presents phytochemicals that target α-synuclein aggregation along with their docking energy scores against the target protein. Among these compounds, the top 2 that meet the threshold criteria for high gastrointestinal absorption will be selected for further validation. Hederagenin (IMPHY007224), a terpenoid derived from Hedera helix, enhances autophagy, promoting the degradation of mutant proteins, and exhibits antidepressant, anti-inflammatory, anticancer, and antioxidant properties [176]. Withanolide A (IMPHY000090), a steroid from Withania somnifera, has anticancer, anti-inflammatory, and antioxidant properties. Additionally, it offers neuroprotective effects by hindering amyloid formation, reducing α-synuclein aggregation, and regulating neurotransmission [177].

In further studies that involves FDA- approved drugs, Amantadine was used as a reference due to its known ability to inhibit the Vps35 protein and regulate physiological processes like protein accumulation, mitochondrial function, and dopamine signaling. Amantadine is an antiviral medication with mild anti-Parkinsonian effects. It functions as a mild, non-competitive antagonist of the NMDA receptor, which results in heightened dopamine release and inhibits dopamine reuptake [178] . FDA-approved drugs, along with Amantadine, were screened using docking analysis. Amantadine achieved a docking score of -4.8 kcal/mol. To filter out inhibitor drugs, a docking threshold of ≤-8 kcal/mol was applied. Compounds meeting this threshold were further analyzed for their ability to penetrate the blood-brain barrier (BBB), resulting in 11 compounds testing positive. The top three ligands selected for further analysis were Irinotecan, Tirilazad, and Alectinib. Additional docking validation using CB-DOCK2 can be conducted to confirm their effective binding inhibition against the target protein.

Table 3.6 & 3.7 shows several physiological properties of the top hit Phytochemicals, this plays a key role in determining both the pharmacokinetic and pharmacodynamic

properties of the drugs. Molecular weight is a key factor in evaluating the drug-likeness of oral drugs, with Lipinski's rule of five suggesting that an optimal molecular weight for good oral drugs is less than 500 Daltons [179]. The partition coefficient, another important physicochemical property, measures a molecule's lipophilicity, indicating whether a drug will reside more in lipid membranes or aqueous media. SWISS ADME employs five predictive models to accurately estimate these properties for the selected compounds [180] . The presence of hydrogen bond donors and acceptors in a compound influences its drug-likeness, as hydrogen bonding creates hydrophobic interactions essential for therapeutic effects [181].

Lipophilicity, indicated by the partition coefficient, should be below 5. Another factor affecting drug bioavailability is the topological polar surface area (TPSA), which ideally should be under 140 Å² for optimal oral bioavailability. In this study, the consensus Log Po/w value, averaged from predictions by five different models in Swiss ADME, is used to assess lipophilicity. Considering all these factors, the lead compound can exhibit excellent physicochemical properties.

The pharmacokinetics profile of a potential drug is a crucial aspect of drug discovery and development. Conducting all these validations in a wet lab is time-consuming and costly, making pharmacokinetic prediction a valuable approach to saving time, resources, and expenses [182]. This helps in identifying potential lead molecules by filtering out unsuitable candidates. Gastrointestinal absorption and brain accessibility predictions are also preliminary tests in determining the fate of a drug candidate. Table 10 shows that all the selected Phytochemicals are having high GI absorption rate and are moderately soluble in water. The model computes the polarity and lipophilicity of small molecules to determine their bioavailability. Bioavailability involves the systemic distribution of a drug's molecules, which influences its therapeutic efficacy, metabolism, and excretion [183] . Blood brain permeability is also one of the major factor that can limit drug action in case of Parkinson disease. Because in this disease we are targeting the drug to brain and thus the selected drug candidate can efficiently cross the blood brain barrier (BBB) for its better efficiency. Therefore it necessitates our study to consider only that potential compounds that can show positive result for BBB test. Table 3.2 and 3.9 illustrates the drug-likeness and pharmacokinetics properties of the selected compound. This study highlights that all the lead compounds display moderate solubility and have high GI absorption rate.

Cytochrome P450 (CYP450) enzymes are essential for drug metabolism. Although there are approximately 50 CYP450 enzymes involved in drug metabolism, around 90% of drug molecules are metabolized by just six key enzymes: CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, with CYP3A4 and CYP2D6 being particularly significant. Drugs that inhibit all of these enzymes may not be metabolized, which could increase the risk of drug toxicity. In our study we want to test the ligands that can show inhibition for at least one of the enzymes mentioned for safe metabolism and excretion [184].

The pharmacological profiling of FDA-approved drugs indicates that Irinotecan and Alectinib exhibit better physicochemical properties than Tirilazad. Both Irinotecan and Alectinib, except for Alectinib, have high molecular weight. All the compounds follow Lipinski's rule and have a bioavailability score of 0.55. Alectinib and Irinotecan demonstrate high solubility beyond the threshold, unlike Tirilazad. Additionally, Tirilazad is an effective inhibitor of cytochrome P450 enzymes.

Tables 3.10 & 3.11 display the toxicity and carcinogenicity predictions for the selected drug candidates, including both phytochemicals and FDA-approved drugs. PkCSM was used to predict AMES toxicity. Toxicity considerations also involve concentration levels, such as Oral rat acute toxicity LD50 and the maximum tolerated dose in humans. The Oral rat acute toxicity LD50 estimates the drug amount causing 50% mortality in test animals, while the maximum tolerated dose in humans determines the highest safe dose. These toxicity predictions provide insight into the safety profiles of the compounds. The Ames mutagenicity test evaluates a molecule's ability to cause mutations in DNA using bacteria, while the human toxicity threshold is established based on the maximum tolerated dose. Carcinogenicity can be checked by CarcinoPred-EL tool and none of the compounds can be carcinogenic. Consequently, all selected phytochemicals and FDA-approved drugs are promising candidates for drug discovery due to their favourable toxicity profiles.

In both studies, we examined the potential of phytochemicals and FDA-approved drugs in targeting the Vps35(D620N) mutation. The results showed that all FDA-approved drugs , two of phytochemicals inhibit α-synuclein aggregation and seven phytochemicals demonstrated strong binding affinity to Vps35(D620N), producing promising outcomes.

CHAPTER-4

CONCLUSION

PD is caused by the deletion of DA neurons, leading to severe motor and non-motor symptoms. Mutations in the Vps35 gene, particularly the D620N mutation, plays an important role in the disease mechanism by disrupting the normal function of the retromer complex, impairing protein recycling, and causing neuronal degeneration. These mutations highlight the importance of Vps35 as a potential therapeutic target. Understanding and targeting Vps35 mutations could pave the way for innovative treatments that address the underlying mechanisms of Parkinson's disease, offering hope for more effective interventions. It attracting global attention due to the absence of treatments that can halt or slow its progression.

Currently, available therapies only address symptoms, and significant research is being conducted to discover better therapies that can decelerate the disease and develop more precise and effective treatments. This study employs two synergistic methods to contribute to this effort: an in-silico approach, including molecular docking and ADMET analysis, to identify novel compounds that can target the mutated Vps35 protein, which is linked to familial dominant forms of Parkinson's disease. The first method focuses on natural inhibitors, while the second utilizes FDA-approved drugs. Both approaches aim to more efficiently target identified protein inhibitors, ensuring greater safety and fewer side effects compared to other inhibitors.

The research methodology involves molecular docking, ADMET analysis, and toxicity and carcinogenicity profiling to evaluate the potential of various Indian medicinal plants and the FDA approved drugs. Results indicate that both the phytochemicals and the FDA approved drugs effectively inhibit the Vps35 protein, a crucial component of the retromer complex and other cellular processes implicated in Parkinson's disease. The study identifies seven phytochemicals compounds—Luteoxanthin, Isowithametelin, Withametelin (Daturilin), Withametelin B, Diosgenin, Daturilinol, and Sarsasapogenin and three FDA approved drugs - Irinotecan, Tirilazad and Alectinib —as inhibitors of the Vps35 protein. These compounds demonstrate the ability to pass the BBB, enhancing their effectiveness in targeting the brain and treating Parkinson's disease.

Further the study identifies Phytochemicals that can inhibit α-synuclein aggregation , an another critical pathological hallmarks in Parkinson disease. Compounds such as Hederagenin and Withanolide A shows high GI absorption, and thus making them suitable for the oral administration targeting α-synuclein aggregation in the gut. It has been shown that the aggregated form of synuclein is initially present in the gut and later on pass to the CNS. Further analysis of these compounds include physiochemical properties, drug-likeness, pharmacokinetic profiling, toxicity and carcinogenicity profiling. This analysis reveals that these two compounds are a promising candidates for drug discovery and development.

Among these, phytochemical Luteoxanthin and Isowithametelin show the highest binding affinity scores with the Vps35 protein and marking them as a promising candidate for therapeutics because they have the ability to pass BBB. The findings suggest that these phytochemical compounds could serve as potent multi-target inhibitors for Vps35 and hold promise as therapeutic candidates for PD. The combination of these phytochemicals for VPS35 in the brain and α-synuclein in the gut can be promising in targeting therapeutics for PD. These findings indicate that plant extracts can exhibit neuroprotective effects by inhibiting α-synuclein oligomerization and fibrillation and may delay the disease progression.

For FDA- approved drugs Irinotecan, Tirilazad and Alectinib can be identified as potent candidates with high docking scores compared to that of the Phytochemicals against the target Vps35(D620N). These FDA- approved compounds have the ability to pass BBB and this ability can enhance the therapeutics effectiveness. Comprehensive analysis of the physiochemical properties, drug-likeness and pharmacokinetics profiling reveals that the phytochemicals performed better than the FDA- approved drugs with least side effects.

The in-silico approach can give promising result in drug discovery and development , but this approach requires further validation through in-vitro assays , these assays could be cell-based assays for assessing cytotoxicity , and the in-vivo studies in animal models for confirming their efficacy and safety. These assays help in practical applications for clinical testing. The primary objective of this study is to develop innovative therapeutics that can decelerate or halt the progression of Parkinson's disease, thereby significantly enhancing the quality of life for patients worldwide.

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Unravelling Parkinson's Disease Therapeutics: Computational Insights into VPS35 via Drug Repurposing

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***Abstract*—Parkinson's disease (PD), is a neurodegenerative movement disorder that is characterized by dopaminergic loss. For getting deeper insights into the molecular and cellular mechanism underlying Parkinson's, studies have now been focused on the genes for the cases that can have genetic origin. The novel cause of a dominant familial form of this disease has been found to defect in the vacuolar protein sorting (VPS35) gene. It is located on chromosome 16. AspD620Asn (D620N), a single missense mutation has been linked to the disease. Deficit of this protein can cause problems with autophagy and lysosomal process. Since VPS35 is involved in Parkinson's disease, it may be a target for therapy. Docking of 3,674 FDA-approved drugs can be done along with reference drugs as a control to find better drugs with greater affinity. Based on their docking scores, three possible candidates were found after docking tirilazad, irinotecan, and alectinib. ADME analysis, which offers details on BBB permeability, water solubility, and bioavailability, was carried out for the validation of these compounds.**

Keywords*— *VPS35, BBB, Parkinson's Disease, Retromer, Swiss ADME, Binding energy

I. Introduction

The second most prevalent neurodegenerative illness is Parkinson's Disease. It may arise due to the degeneration of dopamine-producing neurons in the substantia nigra, which plays a role in the onset of disease. It is the most prevalent illness in middle-aged and senior adults, and it can be recognized by both motor symptoms like bradykinesia and tremors as well as non-motor ones like decreased eyesight, depression, sleeping issues, dementia, and cognitive defects [1]. The loss of dopamine in caudate putamen due to neuronal loss is the cause of motor symptoms [2], [3]. Parkinson's disease can be brought on by several reasons, including genetic, environmental, and aging.

There are currently no disease-modifying treatments available to halt or slow the progression of a disease. It is very crucial to identify and validate new therapeutic drug targets for the management of this disease. Parkinson's disease (PD) typically appears as an unpredictable illness that is most likely brought on by intricate relationships between aging, environmental exposure, and genetic risk.

It is known that the inheritance of mutations in at least 15 genes is the cause of familial variants of Parkinson's disease. Autosomal dominant familial Parkinson's disease with a late onset is brought on by mutations in the VPS35 gene [4]. Dysfunctional VPS35 protein is one of the factors that can cause Parkinson's disease.

Fig. 1. A schematic diagram showing cellular processes that are controlled by VPS35

A. VPS35 as Retromer

Cells regulate many physiological processes through the dynamic process of endosomal trafficking. VPS35 is a vital part of the retromer complex, which helps in recycling and sorting transmembrane cargo proteins retrogradely from endosomes to the plasma membrane and trans-Golgi network [5]. The complex is involved in the trafficking of proteins within cells. It was first discovered to be a component of the yeast retromer complex. It is a subunit of the hetero pentameric complex known as the retromer complex, which is made up of the VPS35, VPS26, VPS29, and SNX-BAR dimer. The retromer complex facilitates the endosome-to-Golgi complex and transmembrane protein cargo transported via endosomes to the plasma membrane [6].

VPS35 forms the main scaffold that binds with the VPS26 at its N-terminus and VPS29 at its C-terminus. It structurally joins forces with VPS26 and VPS29 to form a trimer that is known as the cargo recognition complex (CRC). Next, a dimer of sorting nexins that are members of the SNX-BAR protein family binds to the CRC. RAB7 further facilitates this association. The retromer complex functions as a unit to

organizes endosomes and creates tubular vesicles responsible for sorting cargo either to the plasma membrane for recycling or to the trans-Golgi network (TGN) via retrograde routes. [9].

An accessory protein complex known as the WASH complex can associate with a small portion of the retromer. This binding will then form F-actin patches, that aid in guiding cargo during transit [6]. Through an interaction between the unstructured tail of FAM21 and the C terminus of VPS35, the retromer forms an association with the WASH complex. One of the particular cargoes that are extracted from endosomes and delivered to TGN via this interaction is cation-independent mannose 6-phosphate receptor (CI-MPR) [8].

B. VPS35 in Parkinson

The significance of the roles the retromer complex plays in signal transduction and receptor trafficking retromer dysfunction is most likely to be the primary mechanism underlying the neuronal degeneration brought on by the VPS35 D620N mutation. Impaired WASH complex binding resulting from the D260N mutation causes defects in macroautophagy, which is caused by aberrant trafficking of the autophagy receptor ATG9A. It has been shown that D620N mutations impact AMPA receptor trafficking by overexpressing D620N VPS35 and decreasing VPS35 protein. When the D620N mutation is overexpressed, it causes abnormalities in GluR1 trafficking, which in turn causes downstream defects in synaptic transmission. Interestingly, neurons lacking VPS35 also exhibit abnormal GluR1 and GluR2 trafficking and synaptic transmission. Due to D620N mutation fission factor DLP1 can be overexpressed due to abnormal recycling that can lead to mitochondrial fragmentation.VPS35 is associated with increased LRRK2 activity and decreased recruitment of the WASH complex to endosomes. Additionally, it is implicated in mitochondrial dysfunction, impairment of the autophagy-lysosomal pathway, and disrupted transport of neurotransmitter receptors. [9].

The D620N mutation is also associated, indirectly, with decreased degradation of tau or α-synuclein, as well as other aggregation-prone proteins. The cathepsin D, a lysosomal protease that is connected with α-synuclein degradation can be delivered by CI-M6PR. D620N can normally interact with CI-M6PR, but in cell lines and fibroblast of PD patients, it can alter the sorting mechanism of cathepsin D, leading to an accumulation of α-synuclein [8].

II. METHODOLOGY

A. Preparation of protein structure

The Protein Data Bank (PDB) contains the 3D structure of the VPS35 protein with a resolution of 2.52Å in .sdf (structure data file) format. The VPS26 and VPS29 chain can be downloaded together with the VPS35 from the PDB. PYMOL allows the modification of the VPS35; only the VPS35 chain can remain after the removal of all other chains and water molecules from the structure. The modified VPS35 chain is then saved in the PYMOL window in the format of a .pdb file.

B. Preparation of ligand structure

A docking library approved by the FDA and containing approximately 3,674 drugs can be downloaded from the Drug Bank and used to identify potential inhibitor drugs for VPS35. The open Babel software can be used to convert the ligands from the .sdf format to the .pdbqt format which can be accepted during the docking process. These ligands are converted and then opened in the PyRx's open babel for energy minimization.

C. Molecular docking using PyRx

Compound libraries can be screened using PyRx, a virtual screening tool, against possible drug targets. Autodock and Autodock vina are built into PyRx and can be used for docking. After the ligands and protein structure have been prepared, they can both be seen under the PyRx Autodock tab. Following that, the ligands and protein macromolecules can be chosen with the aid of Vina Wizard. Blind docking was then carried out using Vina Wizard in the PyRx algorithm. Following the successful completion or termination of the docking process, tabulated results displaying the binding affinity of each ligand about the protein macromolecule were obtained; the results and output files were saved in comma-separated values (.csv) format at the selected locations that can be necessary for the analysis of the interaction between the macromolecule and the ligands in the 2-D form that can show every interaction and the hydrogen bonds that can be formed between the ligand and the macromolecule. The interactions were further investigated using Discovery Studio Visualizer and Pymol for 2D and 3D structures.

D. BBB permeability using Swiss ADME

The Swiss ADME tool is a software that can help determine if a selected ligand can cross the blood-brain barrier (BBB) or not. This tool can be accessed for free and predicts the physicochemical, absorption, distribution, metabolism, and elimination properties of molecules. To analyze the ligand's characteristics using Swiss ADME, the canonical SMILES for the ligands can be obtained from PubChem. It provides estimates based on several parameters, such as the P-glycoprotein substrate, Lipinski's rule of five, and the anticipated activity of the central nervous system. It makes predictions; often, tests are necessary to verify a compound's actual capacity to cross the BBB. It is a set of guidelines used in medicinal chemistry to assess if a molecule has the potential to be a drug. To be categorized as orally active, a molecule has to meet certain requirements, including having less than 500 Daltons Molecular Weight, less than five hydrogen bond donors, less than ten hydrogen bond acceptors, and a computed LogP (partition coefficient) value of less than five. All the factors are used to forecast a compound's likelihood of having advantageous pharmacokinetic characteristics when taken orally (http://www.swissadme.ch).

III. RESULT AND DISCUSSION

A. Drug target and BBB permeability

Docking analysis was performed on the 3,674 FDA-approved medications that were obtained from DrugBank. Amantadine is the reference medication for VPS35 that can

be selected from the literature PMID: 28796472. When amantadine is docked with the VPS35 protein, it can yield a binding score that is significantly lower -4.8 kcal/mol—than the compound with the highest affinity. The highest binding affinity comes out to be -10.3 kcal/mol. Based on the observation, we chose compounds with high BBB permeability determined by SWISS ADME analysis and binding energy scores greater than -8.0 kcal/mol. Eleven compounds with BBB penetrant activity are revealed by BBB permeability analysis, and they can be chosen for additional analysis. Table I enlist the interactions and binding affinity score of these 11 compounds, which can be examined by Discovery Studio 2021.

The most negative binding energy is correlated with the most stable interactions of the target-ligand complex. Examining and comprehending these interactions can help in determining how the ligand and protein molecules interact, as well as how the ligand residues attach themselves to the protein molecule. The highest binding score out of all 11 compounds was obtained for Tirilazad against VPS35 of -9.5 kcal/mol followed by Irinotecan and Alectinib. The surface diagram illustrates the three-dimensional interactions of these three ligands with the VPS35 along with the reference drug is shown in Fig. 2.

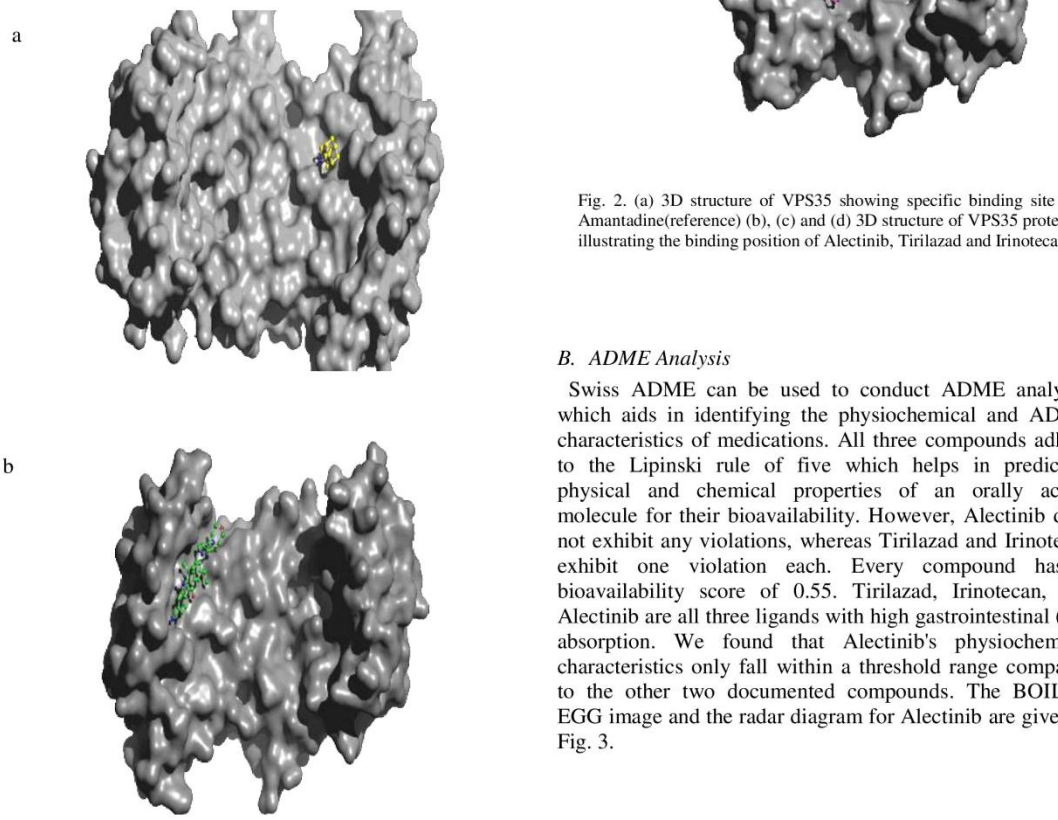

Fig. 2. (a) 3D structure of VPS35 showing specific binding site of Amantadine(reference) (b), (c) and (d) 3D structure of VPS35 protein, illustrating the binding position of Alectinib, Tirilazad and Irinotecan.

B. ADME Analysis

Swiss ADME can be used to conduct ADME analysis, which aids in identifying the physiochemical and ADME characteristics of medications. All three compounds adhere to the Lipinski rule of five which helps in predicting physical and chemical properties of an orally active molecule for their bioavailability. However, Alectinib does not exhibit any violations, whereas Tirilazad and Irinotecan exhibit one violation each. Every compound has a bioavailability score of 0.55. Tirilazad, Irinotecan, and Alectinib are all three ligands with high gastrointestinal (GI) absorption. We found that Alectinib's physiochemical characteristics only fall within a threshold range compared to the other two documented compounds. The BOILED EGG image and the radar diagram for Alectinib are given in Fig. 3.

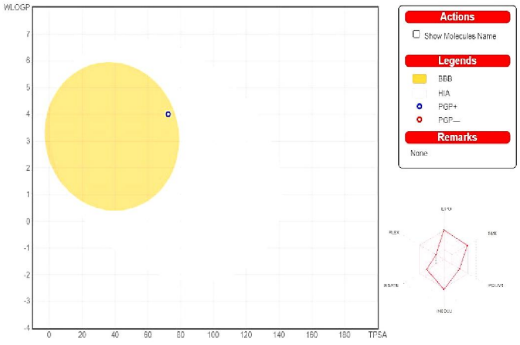

Fig. 3. BOILED EGG image for Alectinib derived by ADME analysis and bioavailability radar diagram of Alectinib showing a radar plot that is positioned entirely within the designated pink area.

VPS35 protein through molecular docking analysis of the FDA-approved drug library. The reference drug used is amantadine, an antiviral drug with antiparkinsonian properties. After docking and ADME analysis, tirilazad, irinotecan, and Alectinib show the best interactions with the target protein and the highest negative binding energy scores. According to the ADME study, Alectinib has better pharmacokinetic qualities than the other two medications. However, more research on these medications can be done to validate the suggested model.

ACKNOWLEDGMENT

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TABLE I. BINDING ENERGY SCORE AND INTERACTIONS OF BBB PERMEABLE LIGANDS ALONG WITH REFERENCE DRUG

S.no	Ligands	Binding free energy (kcal/mol)	Interactions
1.	Tirilazad	-9.5	Pro530, Pro531, Ser489, Gln488, Gly492, Ile495, Arg499, Phe534, Gln538, Tyr537, Gln586, Ala590, Leu589.
2.	Irinotecan	-9.5	Phe534, Gln538, Tyr537, Leu589, Ala590, Ile495, Ala535, Arg499, Gly492, Phe527, Gln488, Pro530, Pro531, Glu593
3.	Alectinib	-9.1	Leu579, Phe534, Pro530, Leu583, Pro531 Ile495, Arg499, His496, Arg526, Phe527, Gln488, Gly492
4.	Ponatinib	-8.6	Pro530, Phe527, gln488, Pro531, Phe534, Tyr537, Gln538, Phe541, Ala590, Gln586, Leu589, Glu593
5.	Mosapramine	-8.4	Phe541, Gln538, Arg499, Phe534, Ala535, Ile495 Pro531, Ala 590, Tyr537, Leu589, Gln586
6.	Piroheptine	-8.4	Phe541, Tyr537, Gln586, Ala590, Leu589, Glu593, Phe534, Gln538
7.	Demegestone	-8.3	Arg499, Gly492, Phe527, Pro531, Gln538, Ile495, Gln488, Phe534, Pro530, Ala535
8.	Astemizole	-8.2	Leu579, Pro530, Phe534, His496, Arg499, Gly492, Ile495, Pro531, Gln488, Phe527, Arg526, Leu575, Glu578
9.	Cyproheptadine	-8.2	Ala590, Phe541, Leu589, Glu593, Tyr537, Gln538, Phe534, Gln586,
10.	Ketoconazole	-8.2	Gln538, Pro530, Pro531 Phe534, Gln586, Phe541, Tyr537, Ala590, Leu589
11.	Metergoline	-8.2	Phe541, Gln538, Tyr537, Ala590, Glu593, arg637, Leu589, Gln586, Phe 534.
12.	Amantadine (Reference)	-4.8	Gln586, Phe541, Phe534, Gln538, Ala590, Tyr537

IV. CONCLUSION

The top 11 compounds exhibit binding at the active sites of the VPS35 protein, having the highest binding affinities and being BBB positive. Molecular docking revealed three compounds that target VPS35 with maximal inhibition or binding and a better binding affinity score than the reference medications listed in the literature. These compounds were also BBB permeable. To effectively treat Parkinson's disease, it is critical to identify new therapeutic opportunities. In this work, we propose inhibitors of the

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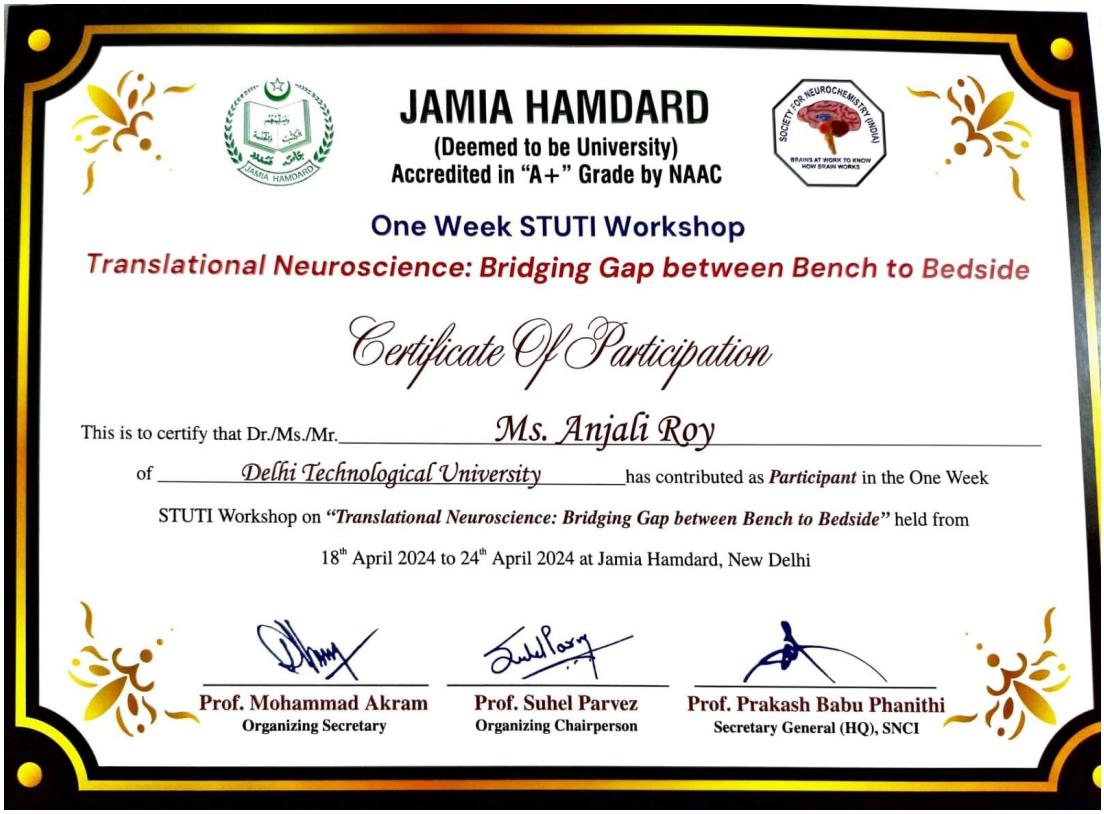

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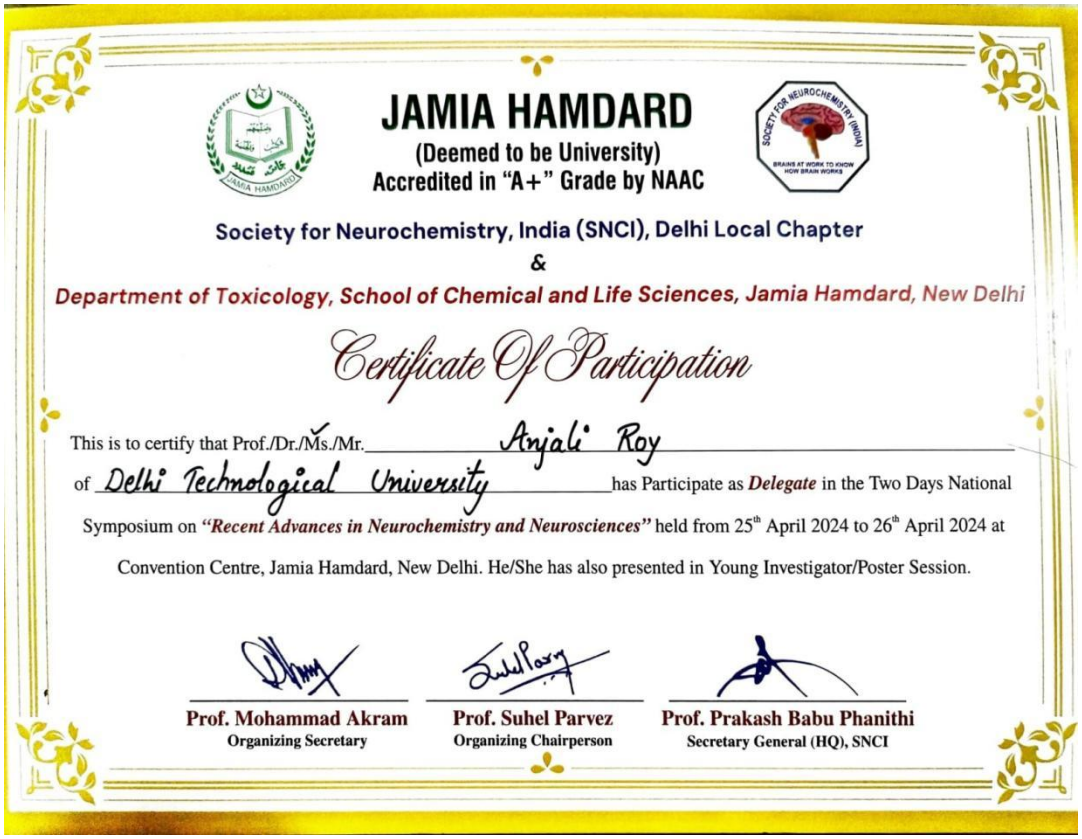

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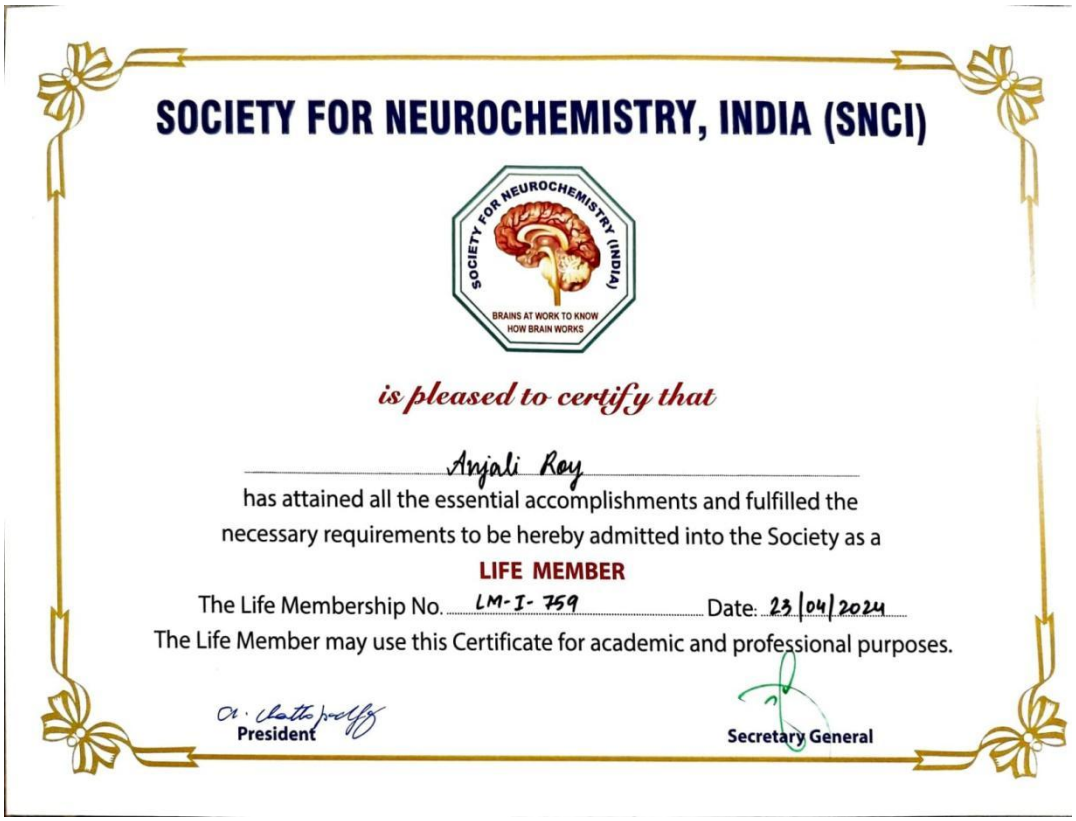

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CBSE (Class X)	2016	Convent of Gagan Bharti Sr. Sec. School	8.6 CGPA

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- Participated in the "National Workshop on Immuno-Biology Techniques and Their Applications" held from February 13 - February 25, 2023 organised by Centre for Innovation in Infectious Disease Research, Education and Training (CIIDRET) University of Delhi South Campus and Delhi School of Skill Enhancement and Entrepreneurship Development (DSSEED-IoE), University ofDelhi.

- Attended "National Workshop on Techniques for Manipulation of Nucleic Acids for Application in Genomics" organised by DBT-Supported Genomics Facility, Centre for Innovation in Infectious Disease Research, Education and Training (CIIDRET) University of Delhi South Campus and Delhi School of Skill

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CONFERENCES

- Attended and presented poster in "3rd International Conference on Antimicrobial Resistance, Novel Drug Discovery and Vaccine Development: Challenges and Opportunities" held on March 18 - March 20, 2024.
- Attended and presented poster in Two Days National Symposium on "Recent Advances in Neurochemistry and Neurosciences" held from April 25 - April 26,organised by Department of Toxicology, School of Chemical and Life Sciences,Jamia Hamdard, New Delhi and Society for Neurochemistry, India (SNCI), Delhi Local Chapter.
- Participated and Presented paper titled " Unravelling Parkinson's Disease Therapeutics: Computational Insights into VPS35 via Drug Repurposing" at 2nd IEEE International Conference on Knowledge Engineering and Communication Systems (ICKECS) 2024 technically co- sponsered by IEEE Bangalore Section, Chikkaballapur, Karnataka, India organized at SJC Institute of Technology.

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