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In Silico DOCKING STUDIES IN IDENTIFICATION OF PHYTOCOMPOUNDS FROM SELECTED MEDICINAL PLANTS AS POTENT INHIBITORS OF ALPHA SYNUCLEIN AGGREGATION IN TREATMENT OF PARKINSON'S DISEASE

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Abstract: Parkinson's disease is a neurodegenerative disorder that have emerged as among the serious health problems of the 21st century. One of the Pathological hallmarks of PD is alpha-synuclein (α-Syn) self-aggregation, the existence of intracytoplasmic inclusion bodies called Lewy bodies (LBs) and Lewy neurites (LNs) causes PD, which is a cause of neuronal death. The medicament currently used to treat Parkinson's disease have limited efficacy and are associated with adverse effects. Natural products are one of the most indispensable to the continuance of life and conservative sources of medicines for treating neurological problems. This research was conducted with the overarching aim of discovering the potential phytocompounds from 22 locally available medicinal plants which is known for their Anti-Inflammatory, Anti-Oxidant, Antitumour, Anti-neurotoxic, Neuroprotective activity etc… which could be used to treat Parkinson's Disease. IMMPAT and PubChem Database were used to retrieve the 3D structures of the phytocompounds. To analyse the neuroprotective effects of the phytocompounds, an extensive *in-silico* studies starting with molecular docking against Human Micelle-bounded Alpha Synuclein as a putative target for Parkinson's disease with 856 compounds were conducted using PyRx software. The findings were compared with 4 standard drugs used for Parkinson's disease using Discovery Studio 2021. The Lipinski Rule of Five, ADMET and CYP properties were assessed using SwissADME. The Phytocompounds Diosgenone,4-dimethyl-, Tigogenin and Ecliptalbine showed a strong affinity when compared with the standard drugs. Interaction of ligands with Alpha synuclein receptor showed a very strong affinity of -7.9 Kcal/mol for Diosgenone,4-dimethyl-, -7.6 Kcal/mol for Tigogenin and -7.4 Kcal/mol for Ecliptalbine respectively. By analysing the ADMET and CYP properties of these compounds, it is suggested that Tigogenin has the potential to act as a Lead compound against α-Syn aggregation. Further the *in vitro* and *in vivo* studies are suggested to confirm the promising therapeutic capability.

Index Terms **- Neuroprotective, PyRx, Parkinson's disease, Phytocompounds, ADMET, CYP properties, drug-likeliness.**

I. INTRODUCTION

Parkinson's Disease (PD) is a progressive Neurodegenerative complaint of the Central Nervous system which has adverse effects both the motor system and non-motor systems [37]. PD is reported as a loss of dopaminergic neurons located in the substantia nigra (SN) and affects 1- 2 of people the time of 60 and older [39]. The finding of PD genes has led to the hypothesis that misfolding of protein & dysfunction of ubiquitin proteasome pathway, mitochondrial dysfunction and oxidative stress are causes for PD pathogenesis [1]. The cause of this cell death is poorly understood, but involves the aggregation of the protein alpha-synuclein into Lewy bodies within the neurons. The symptoms usually occur slowly, and as the disease worsens, non-motor symptoms become more common [2]. Early symptoms are tremor, rigidity, slowness of movement, and difficulty with walking. Cases may also rise with cognition, gesture, sleep, and sensitive systems [38]. No cure for PD is known; treatment aims to reduce the goods of the symptoms. Original treatment is generally with the specific's levodopa (L- DOPA), MAO- B impediments, or dopamine agonists [6]. As the disease progresses, these specifics come less operative, while at the same time producing a side effect marked by involuntary muscle movements. At that time, specifics may be exercised in combination and doses may be increased. Thus, the search for new therapeutic agents with few side effects is essential.

Upcoming diagnostic techniques and encouraging neuroprotective pharmacological agents are becoming a reality enabling the next level in PD therapy [2]. A 140 amino acid protein, α-synuclein is usually located in presynaptic terminals [3,4]. Alpha-synuclein represents the most occurring protein in Lewy bodies (LB), cytoplasmic inclusions found in PD and in LB dementia (LBD), which have a poorly understood physiology [5]. The synuclein family has three members, α-synuclein, β-synuclein, and γ-synuclein, ranging from 127 to 140 amino acids, with about 55 to 62% of homologous sequences, and where α and β have an identical carboxy-terminal domain. These proteins are generally found in nerve terminals, near to synaptic vesicles; β-synucleins are present in almost every nerve cell [7]. α-synuclein was first isolated from TORPEDO CALIFORNIA CHOLINGERIC Synaptic vesicles and later as the non-amyloid component of plaques from Alzheimer diseased brain [8]. Its main function is still unknown but it is found to be the major component of Lewy bodies [LB] which are neuronal cytoplasmic inclusions of aggregated protein that are characteristic hallmarks of idiopathic and familial forms of PD [9].

Factors that have impact on α-synuclein abnormalities, inheritable factors (protein gene, PARK3, and PARK4 locus mutations) and environmental aspects (oxidative damages) often lead to errors in the ordering and conformation of α-synuclein filaments [10]. Latest studies report a mutation of alanine to threonine at position 53 of the protein gene causes an unusual and familial form of PD in four families [11]. The detection of this mutation in autosomal dominant families of inherited Parkinson's led to the discovery of a new target for PD pathology.

Olanow and Brundin [12] gave evidence of α-synuclein activity in prion-like proteins acting in PD, thus suggesting upcoming studies for the development of inhibitors. Recent studies have turned up that a doubling or tripling of the α-synuclein gene leads to a similar type of PD [13,14]. Amino acid residue 64-100 of alpha synuclein is described as the binding region responsible for self-association.

Oxidative stress is thought to be an important factor in PD due to the destructive effect of free radicals and enhanced fibrillation of α-synuclein which is toxic to neuronal cells [15]. Antioxidants represent a huge class of potential therapeutic agents for neurodegenerative diseases. These compounds are used to prevent oxidation of other molecules reducing overall free radical levels and cellular oxidative stress [8]. Various research report has indicated that a range of pure compounds derived from herbal materials, herbal extracts/ fractions & herbal formulations are effective on *in vitro/ in vivo* PD models [16]. Use of these natural products against PD has intensified in recent years, chiefly compounds derived from plants, since they are known to have fewer side effects than synthetic compounds [17,18]. These advancement in the treatment of PD provide the disease a chance to be administered potentially, leading to symptom control and development of patient quality of life, often for decades after onset of the disease [5].

With the help of Computer aided drug design (CADD), potent compound that has the ability to bind to the active site of alpha synuclein can be identified effectively. Nowadays, molecular docking studies are very frequently used in modern drug design molecules [19]. Docking was performed between the phytocompounds and the target protein Human Micelle-bounded Alpha Synuclein using PyRx Version 0.8 software. About 1087 phytocompounds from 22 locally available medicinal plants which is known for their Anti-Inflammatory, Anti-Oxidant, Antitumor, Anti-neurotoxic, Neuroprotective activity etc. are taken. The binding effect of the retrieved phytocompounds with the specified target was predicted using molecular docking. The pharmacokinetic (Absorption, Distribution, Metabolism, And Excretion) toxicity properties of the screened phytocompounds were also studied [27]. Based on the above studied parameters, obtained results will compare the potential of the phytocompounds with Synthetic drugs which will provide insights in understanding the activities of these compounds as inhibitors and potent compound can be introduced into further trials.

II. MATERIALS AND METHODS

2.1 Ligand Selection:

Using the literature and IMMPAT Database [36] around 1087 phytochemical compounds were selected from 21 medicinal herbs known for its Anti-Inflammatory, Anti-Oxidant, Antitumour, Anti-neurotoxic, Neuroprotective etc… The plants including *Abutilum Indium, Aegle marmelos, Aerva Lanata, Anisomeles malabarica, Argemone mexicana, Bacopa monneri, Basella rubra, Boerhavia diffusa, Bryophyllum pinnatum, Cheilocostus speciosus, Cichorium intybus, Cissus quadrangularis, Cypernus rotundus, Datura metal, Eclipta prostrasta, Elettaria cardamomum, Gingko biloba, Hemidesmus indicus, Hygrophila auriculata, Tribulus terrestris* and *Withania somnifera* are used to inhibit Alpha synuclein receptor. The 3D structures of these compounds with their physical and chemical properties were retrieved from the PubChem compound database [20]. The phytochemical compounds were downloaded in the SDF format and used for recurrent study.

2.2 Target Protein Selection:

From the previous studies [5,35] and as per the literature, it was determined that one of the potential drug targets for the Parkinson's disease is Human Micelle-bounded Alpha Synuclein (PDB ID: 1XQ8) [22]. The NMR solution structure of Human Micelle-bounded Alpha Synuclein (PDB ID: 1XQ8) [21] and its 3D structure were obtained from the Protein Data Bank (PDB) (https://www.rcsb.org). The protein targets were prepared by removing the unwanted hetero atoms and water molecules from it. Hence, the target protein was prepared using Discovery Studio 2021 Client.

2.3 Preparation of Target Proteins:

Preparation of the target proteins involves with the addition of polar hydrogen atoms, addition of charges and removal of any unwanted substances which involves in the reaction. The miscellaneous substances like water molecules and the hetero atoms are removed and the polar hydrogens were added. Further, energy minimization of the target structure was performed after adding hydrogen atoms to obtain a properly optimized position of side chain atoms and hydrogen atoms using Discovery Studio 2021 Client software.

2.4 Lipinski Rule of Five Analysis for The Screened Compounds:

An online web tool "Lipinski Rule of Five, a supercomputing Facility for Bioinformatics and Computational Biology, IIT Delhi" (http://www.scfbio-iitd.res.in/ software/drug design/lipinski. jsp) was used to retrieve the information about the drug likeness of the drug. It helps to differentiate between drug like and non-drug like molecules [23]. It anticipates the high chances of success or failure due to drug likeness for molecules complying with 2 or more of the following rules:

- Molecular mass less than 500 Dalton
- High lipophilicity (expressed as Log P less than 5)
- Less than 5 hydrogen bond donors
- Less than 10 hydrogen bond acceptors
- Molar refractivity should be between 40-130

The Lipinski Rule of Five was assessed for the retrieved compounds and further moved to Docking studies.

2.5 Molecular Docking Studies:

PyRx software (Version 0.8, with Virtual Screening and Drug discovery features) was used to perform molecular docking with the target protein (PDB ID: 1XQ8) and the phytochemical compounds respectively [24]. Using the software, the target proteins were further prepared for molecular docking studies. The protein molecules were converted into (. pdbqt) file format. The Open Babel option in the PyRx was used to import the ligands. Using the Open Babel feature, Energy of ligands were minimized and the ligands were converted in (. pdbqt) file format. Using the Vina Wizard feature in the software which remains in-built in the PyRx is used to import the macromolecule and the molecular grid was built and the docking studies were performed [24]. The binding affinities were exported as an Excel file (.csv file format). The results were analyzed using the Discovery Studio 2021 Client and the 2D and the 3D receptor-ligand interactions were taken. In the results, the lowest binding affinity indicates the best fit.

2.6 ADMET and CYP Properties:

SwissADME was used to assess the ADMET and CYP characteristics of the top 10 phytochemical compounds for the target protein [25]. The compounds with the best interaction and passed Lipinski criteria were examined using BBB (Blood Brain Barrier permeability), HIA (Human Intestinal Absorption), PGP (P-Glycoprotein), XLogP3, TPSA (Topological Polar Surface Area), Log S, Fraction Csp3, Rotatable bonds, CYP enzyme inhibitor properties, Skin permeation, and Bioavailability score.

III. RESULTS

3.1 Ligand and Target Protein Selection:

The PubChem Compound Database was used to retrieve the 3D structure of the ligands and the 3D structure of the target proteins were retrieve from the Protein Data Bank Database (PDB) (https://www.rcsb.org) [20]. The NMR solution structure of Human Micelle-bounded Alpha Synuclein (PDB ID: 1XQ8) and its 3D structure was retrieved. The 3D structure of the target protein is shown in fig 1.

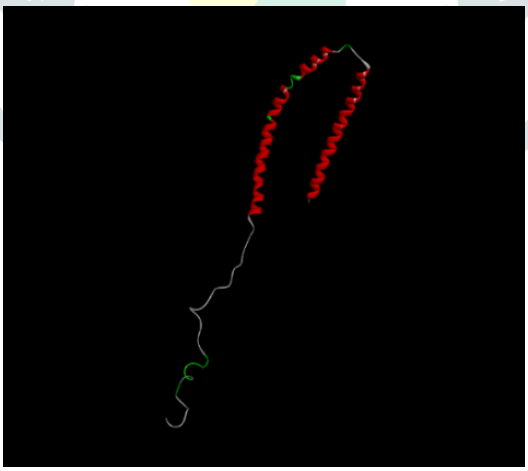

Figure 1: Structure of Human Micelle-bounded Alpha Synuclein (PDB ID: 1XQ8)

3.2 Lipinski Rule of Five Analysis:

Lipinski rule of Five was performed to analyse the Drug likeness properties of the 1087 phytocompounds. Among 1087 phytocompounds, 854 compounds showed drug likeness property and they were further moved to molecular docking studies. The Lipinski Rule of Five for the top 10 Phytocompounds were represented in the Table 3.2

Table 3.2: Lipinski Rule of Five for Top 10 Phytocompounds.

S. No	Name of the Compound	Molecular mass less than 500Da	High lipophilicity	Less than 5 H bond donors	Less than 10 H bonds acceptor	Molar refractivity (40-130)	Total Score (out of 5)
1	Somniferine	No	Yes	Yes	Yes	No	3
2	Bilobetin	No	Yes	Yes	Yes	Yes	4
3	Isoginkgetin	No	Yes	Yes	Yes	Yes	4
4	Diosgenone,4-dimethyl-	Yes	Yes	Yes	Yes	No	4
5	Neogitogenin	Yes	No	Yes	Yes	No	3
6	Chaetoglobosin U	Yes	Yes	Yes	Yes	Yes	5
7	Tigogenin	Yes	Yes	Yes	Yes	No	4
8	Protopine	Yes	Yes	Yes	Yes	Yes	5
9	Chlorogenin	Yes	Yes	Yes	Yes	Yes	5
10	Ecliptalbine	Yes	Yes	Yes	Yes	No	4

3.3 Docking Studies:

Docking studies were performed for the phytocompounds for the selected 21 medicinal plants and the target protein Human Micelle-bounded Alpha Synuclein using PyRx 0.8 software to identify the potential drug candidate for the treatment of Parkinson's Disease (PD). To achieve this, 854 phytocompounds which passed the Lipinski Rule of Five were interacted with the target protein using this software. The results were exported as (.CSV file) Excel file through this software and the binding affinity value was noted. The Receptor-Ligand interactions of the phytocompounds were analysed using Discovery Studio 2021. From the results, the following Top 10 showed a very good interaction with the target protein. Further some of the standard drugs used for the treatment of Parkinson's Disease such as Clozapine, Tolcapone, Benztropine and Amantadine were also taken to find the interaction and effectiveness with the target protein. The Binding affinity, interacting residues and their bond lengths were tabulated in Table 3.3.

Table 3.3: Phytocompounds and Synthetic Drugs with their Binding Affinity, Interacting Residues and its Bond Length (in A°)

S. No	NAME OF THE COMPOUND	PUBCHEM ID	PLANT NAME (For synthetic drugs – usage)	BINDING AFFININTY (kcal/mol)	NO. OF BONDS	INTERACTING RESIDUCES (in the receptor protein)	BOND LENGTH (A°)
Phytocompounds to 1xq8							
1	Somniferine	14106343	*Withania somnifera*	-8.3	10	LYS A:45 TYR A:39 TYR A:39 LYS A:43 LYS A:43 VAL A:40 GLY A: 36 GLU A:35 LEU A:38 GLY A:41	2.44 4.79 5.39 2.70 3.09 2.60 3.63 3.36 3.69 2.97
2	Bilobetin	5315459	*Ginkgo biloba*	-8	11	GLU A:35 GLU A:35 LEU A:38 GLY A:36 LYS A:43 LYS A:43 LYS A:32 VAL A:48 LYS A:45 LYS A:45 GLY A:41	2.27 2.46 2.11 3.48 4.88 4.51 2.02 3.82 5.45 2.51 2.42

3	Isoginkgetin	5318569	*Ginkgo biloba*	-7.9	10	VAL A:43 VAL A:43 LYS A:32 LYS A:48 LYS A:48 GLU A:35 GLY A:36 GLY A:36 GLY A:41 LYS A:45	4.88 4.50 1.99 3.80 4.12 2.24 2.80 3.50 2.42 5.44
4	Diosgenone,4-dimethyl-	249449	*Cheilocostus speciosus*	-7.9	2	LYS A:45 GLY A:36	4.34 2.64
5	Neogitogenin	12304409	*Tribulus terrestris*	-7.8	2	LYS A:32 LYS A:32	2.80 2.63
6	Chaetoglobosin U	11570022	*Ginkgo biloba*	-7.7	7	TYR A:136 SER A:129 PRO A:128 GLU A:126 MET A:127 TYR A:125 TYR A:125	5.38 2.92 2.48 2.32 2.18 5.41 4.56
7	Tigogenin	99516	*Cheilocostus speciosus*	-7.6	4	LYS A:32 LYS A:32 TYR A:39 TYR A:39	3.81 5.11 5.14 5.40
8	Protopine	4970	*Argemone mexicana*	-7.6	5	LYS A:32 GLU A:35 GLY A:36 TYR A:39 TYR A:39	2.29 4.17 3.49 5.31 4.86
9	Chlorogenin	12303065	*Tribulus terrestris*	-7.6	3	LEU A:38 GLU A:35 LYS A:43	2.73 1.91 5.15
10	Ecliptalbine	10692897	*Eclipta prostrasta*	-7.4	5	LYS A:43 LYS A:43 LYS A:45 VAL A:48 VAL A:48	2.90 4.74 5.29 4.17 3.84
Standard Drugs (USED TO TREAT PD) to 1xq8							
1	Clozapine	135398737	Anti-parkinson drug (Neuroleptic Drug)	-6.1	5	TYR A:39 TYR A:39 VAL A:40 VAL A:40 LYS A:43	5.22 4.93 2.10 5.19 4.21
2	Benztropine	1201549	Anti-parkinson drug (Anticholinergic Drug)	-5.8	7	TYR A:39 VAL A:40 LYS A:43 LYS A:43 LYS A:45 LYS A:45 VAL A:48	4.87 4.90 5.16 3.63 5.43 2.66 4.99
3	Tolcapone	4659569	Anti-parkinson drug (COMT Inhibitor)	-5.8	8	GLU A:130 GLU A:126 GLU A:126 GLU A:126 MET A:127 SER A:129 GLY A:132 TYR A:125	2.71 2.12 2.83 4.09 2.58 2.55 2.33 4.97

4	**Amantadine**	**2130**	**Anti-parkinson drug (Unspecified)**	**-4.2**	**7**	**TYR A:39 VAL A:40 VAL A:40 VAL A:40 VAL A:40 LYS A:43 LYS A:43**	**5.30 2.90 5.28 2.44 1.76 4.18 1.89**

From the results (Table 3.3), among 854 phytocompounds, these 10 compounds showed very good results with the target protein. To be noted that the phytocompound Somniferine showed excellent binding affinity (-8.3 Kcal/mol) with the amino acid residues LYS A:45, TYR A:39. TYR A:39, LYS A:43, LYS A:43, VAL A:40, GLY A: 36, GLU A:35, LEU A:38 and GLY A:41 of the target protein. The phytocompound Bilobetin also provided a strong binding affinity of -8 Kcal/mol with the amino acid residues GLU A:35, GLU A:35, LEU A:38, GLY A:36, LYS A:43, LYS A:43, LYS A:32, VAL A:48, LYS A:45, LYS A:45 and GLY A:41. The binding affinity -7.9 Kcal/mol was observed between the phytocompound Isoginkgetin and the amino acid residues VAL A:43, VAL A:43, LYS A:32, LYS A:48, LYS A:48, GLU A:35, GLY A:36, GLY A:36, GLY A:41 and LYS A:45. The phytocompound Diosgenone,4-dimethyl- provided a good affinity of -7.9 Kcal/mol with the amino acid residues LYS A:45 and GLY A:36. The phytocompound Neogitogenin gave a good affinity of -7.8 Kcal/mol with the amino acid residues LYS A:32 and LYS A:32. The phytocompound Chaetoglobosin U gave a good affinity of -7.7 Kcal/mol with the amino acid residues TYR A:136, SER A:129, PRO A:128, GLU A:126, MET A:127, TYR A:125, TYR A:125, LYS A:32, LYS A:32, TYR A:39 and TYR A:39. The phytocompound Tigogenin had a good interaction with the protein by providing good affinity of -7.6 Kcal/mol with the residues LYS A:32, LYS A:32, TYR A:39 and TYR A:39. The phytocompound Protopine and chlorogenin gave a good affinity of -7.6 Kcal/mol each. The interacting residues for Protopine are LYS A:32, GLU A:35, GLY A:36, TYR A:39, TYR A:39 and for Chlorogenin is LEU A:38, GLU A:35 and LYS A:43. The phytocompound Ecliptalbine had lowest affinity among the top 10 and its residues are LYS A:43, LYS A:43, LYS A:45, VAL A:48 and VAL A:48.

Moreover. The binding affinity of the synthetic drugs Clozapine, Benztropine, Tolcapone and Amantadine were -6.1 Kcal/mol, -5.8 Kcal/mol, -5.8 Kcal/mol and -4.2 Kcal/mol respectively. The interacting residues for Clozapine were TYR A:39, TYR A:39, VAL A:40, VAL A:40 and LYS A:43. For Benztropine, the interacting residues were TYR A:39, VAL A:40, LYS A:43, LYS A:43, LYS A:45, LYS A:45 and VAL A:48. For Tolcapone, GLU A:130, GLU A:126, GLU A:126, GLU A:126, MET A:127, SER A:129, GLY A:132, TYR A:125 and for Amantadine TYR A:39, VAL A:40, VAL A:40, VAL A:40, VAL A:40, LYS A:43, LYS A:43 were the interacting residues.

3.4 PHARMACOKINETIC PROPERTIES OF THE SCREENED COMPOUNDS:

3.4.1 ADMET properties:

In the present study, ADMET properties were tested for the best 10 interacted phytocompounds and standard drugs Clozapine, Benztropine, Tolcapone and Amantadine using SwissADME and the results were tabulated in Table 3.4.1.

Table 3.4.1: ADMET properties of phytocompounds and Synthetic Drugs

S. No	PubChem (CID)	Compound Name	Lipinski Rule (#violations)	BBB	HIA	PGP Substrate	XLOG P3	TPSA (A^0)	Log S (ESOL)	Fraction Csp3	Rotatable Bonds
Phytocompounds to 1XQ8											
1	**14106343**	**Somniferine**	1	No	High	Yes	2.61	100.93	-5.26	0.47	3
2	**5315459**	**Bilobetin**	1	No	Low	No	5.36	170.8	-6.96	0.03	4
3	**5318569**	**Isoginkgetin**	1	No	Low	No	5.69	159.8	-7.17	0.06	5
4	**249449**	**Diosgenone,4-dimethyl-**	1	No	High	No	6.31	55.76	-6.63	0.83	0
5	**12304409**	**Neogitogenin**	1	Yes	High	Yes	5.52	58.92	-6	1	0
6	**11570022**	**Chaetoglobosin U**	1	No	High	Yes	3.62	111.79	-5.44	0.53	2
7	**99516**	**Tigogenin**	1	Yes	High	No	6.49	38.69	-6.51	1	0
8	**4970**	**Protopine**	0	Yes	High	Yes	2.79	57.23	-4.13	0.35	0
9	**12303065**	**Chlorogenin**	1	Yes	High	Yes	5.15	58.92	-5.77	1	0
10	**10692897**	**Ecliptalbine**	1	No	High	No	6.05	53.35	-6.21	0.74	2

Standard drugs to 1XQ8											
1	**135398737**	**Clozapine**	0	Yes	High	Yes	3.23	30.87	-4.22	0.28	1
2	**1201549**	**Benztropine**	0	Yes	High	No	4.48	12.47	-4.69	0.43	4
3	**4659569**	**Tolcapone**	0	No	High	No	3.3	103.35	-3.86	0.07	3
4	**2130**	**Amantadine**	0	Yes	High	No	2.44	26.02	-2.31	1	0

Note: Obey Lipinski: yes, no violations - good, Blood-Brain Barrier: Yes, denotes recommend, Human Intestinal Absorption (HIA): High indicates recommendable drug, PGP- (Molecules predicted not to be excreted from the CNS by P-glycoprotein): XLOGP3 score between 0.7 and +5.0 indicates acceptable lipophilicity. Polarity: Good is defined as TPSA between 20 and 130 Å 2. Water Solubility (Log S scale: Insoluble = -10, Poor = -6, Moderate = -4, Soluble = -2, Very = 0, Highly): Log S value less than 6 indicates good, Saturation (Fraction Csp3): Good saturation is defined as a fraction of carbons in the sp3 hybridization that is not less than 0.25. Flexibility (Rotatable Bonds): Good if there are no more than 9 rotatable bonds. [26]

From the results, all the best interacted phytocompounds and also the synthetic drugs obeyed the Lipinski Rule of Five. Most of the compounds did not cross Blood-Brain Barrier (BBB). The phytocompounds Somniferine, Diosgenone,4-dimethyl, Neogitogenin, Chaetoglobosin U, Tigogenin, Protopine, Chlorogenin, Ecliptalbine and all synthetic drugs had a High Intestinal Absorption (HIA). Among the top 10 compounds, Bilobetin, Isoginkgetin, Diosgenone 4-dimethyl, Tigogenin and Ecliptalbine are predicted not to be effluated from the CNS by P-Glycoprotein. Among the 10 compounds, XLogP3 value of three compounds were within the range and remaining compounds were above the range. TPSA (Topological Polar Surface Area) and Log S value of the most of the compounds were within the limit. The Fraction Csp3 value of Bilobetin and Isoginkgetin were less the 0.25 and the value of other compounds were within the limit. Rotatable bonds of all the compounds were in the acceptable range.

BOILED EGG:

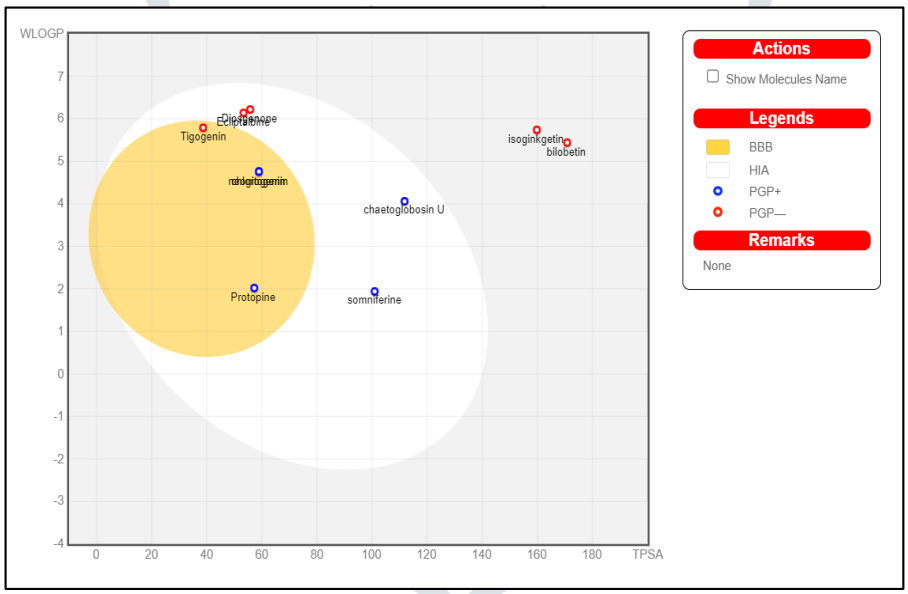

Figure 3.4.1 a) ADMET Properties – Boiled Egg for the Top 10 Phytocompounds

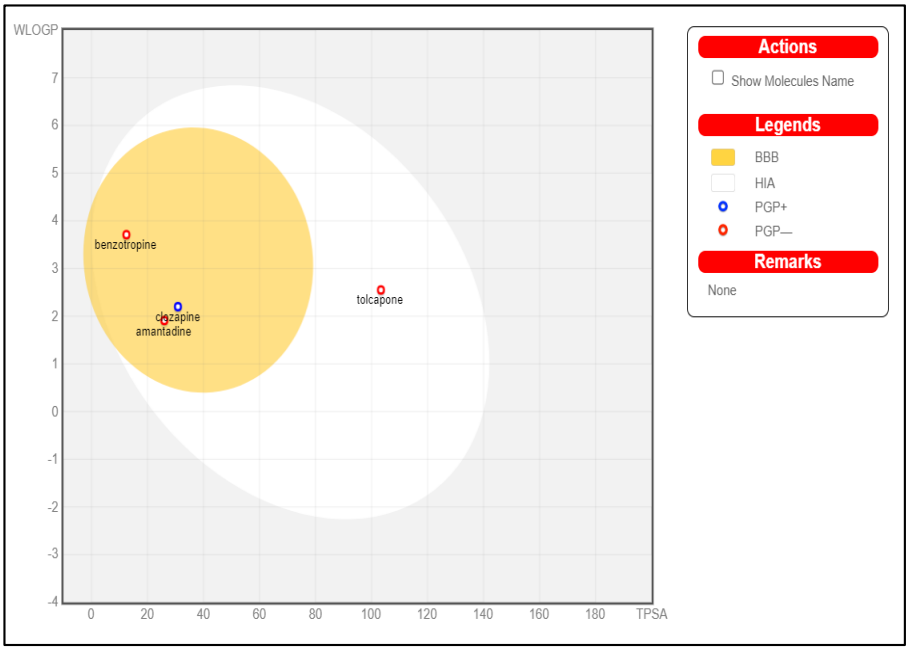

Figure 3.4.1 b) ADMET Properties – Boiled Egg for the Synthetic compounds

Note:
BBB: Points located in BOILED-Egg's yolk are molecules predicted to passively permeate through the blood-brain barrier.
HIA: Points located in BOILED-Egg's white are molecules predicted to be passively absorbed by the gastrointestinal tract.
PGP+: Blue dots are for molecules predicted to be effluated from the central nervous system by the P-glycoprotein.
PGP-: Red dots are for molecules predicted not to be effluated from the central nervous system by the P-glycoprotein. [26]

From the results of the Boiled Egg image of the phytocompounds (Fig 3.4.1 a), the compounds Tigogenin, Chlorogenin, Neogitogenin and Protopine are located in the Egg Yolk region, which means that the compounds can passively permeate through the Blood-Brain Barrier. The compounds Diosgenone 4-dimethyl, Ecliptalbine, Chaetoglobosin U and Somniferine are located in the Egg-White region, which means they are passively absorbed by the Gastrointestinal tract. The Compounds Isoginkgetin and Bilobetin were located outside the Egg, which means that they are poorly absorbed by the GI tract. The compounds, Bilobetin, Isoginkgetin, Diosgenone 4-dimethyl, Tigogenin and Ecliptalbine are predicted not to be effluated from the CNS by P-Glycoprotein.

3.4.2 CYP properties:

The majority of the substances do not inhibit the CYP450 enzymes or cause any negative side effects, according to the results of CYP characteristics (Table 3.4.2). The Phytocompounds Bilobetin and Isoginkgetin inhibits the CYP450 enzyme CYP2C9. The phytocompound Protopine inhibits CYP450 Enzymes CYP1A2, CYP2C9, CYP2D6 and CYP3A4. The phytocompound Ecliptalbine inhibits CYP450 enzyme CYP1A2. All other phytocompounds does not inhibit any CYP450 enzyme. The value of log Kp (Skin Permeant) is acceptable for all compounds and A Bioavailability Score (ABS) satisfies the rule of five and is acceptable for all of the compounds.

Table 3.4.2: CYP Properties of the phytocompounds and synthetic drugs

S. No	PubChem ID	Compound Name	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor	Log K_p (Skin Permeation) (cm/s)	A Bio-Availability Score (ABS)
Phytocompounds									
1	**14106343**	**Somniferine**	No	No	No	No	No	-8.16	0.55
2	**5315459**	**Bilobetin**	No	No	Yes	No	No	-5.86	0.55
3	**5318569**	**Isoginkgetin**	No	No	Yes	No	No	-5.72	0.55
4	**249449**	**Diosgenone,4-dimethyl-**	No	No	No	No	No	-4.59	0.55
5	**12304409**	**Neogitogenin**	No	No	No	No	No	-5.02	0.55
6	**11570022**	**Chaetoglobosin U**	No	No	No	No	No	-6.95	0.55

7	**99516**	**Tigogenin**	No	No	No	No	No	-4.23	0.55
8	**4970**	**Protopine**	Yes	No	Yes	Yes	Yes	-6.47	0.55
9	**12303065**	**Chlorogenin**	No	No	No	No	No	-5.28	0.55
10	**10692897**	**Ecliptalbine**	Yes	No	No	No	No	-4.5	0.55
Standard Drugs									
1	**135398737**	**Clozapine**	Yes	No	No	Yes	Yes	-6	0.55
2	**1201549**	**Benztropine**	No	No	No	Yes	No	-4.99	0.55
3	**4659569**	**Tolcapone**	No	No	Yes	No	Yes	-5.62	0.55
4	**2130**	**Amantadine**	No	No	No	No	No	-5.49	0.55

Note:

Yes, which indicates that the substance inhibits the CYP450 enzymes and causes unexpected negative effects; No indicates that the substance has no negative effects and does not inhibit the CYP450 enzymes; The more negative the log Kp is the molecule is poorly skin permeable; ABS 0.55 indicates that it satisfies the rule of five, whereas 0.17 indicates that it does not. [26]

From the results, the phytocompounds Somniferine, Diosgenone,4-dimethyl-, Neogitogenin, Chaetoglobosin U, Tigogenin and Chlorogenin did not inhibit any CYP450 enzyme. Compounds with a high log Kp negative value have limited ability to penetrate skin. According to this statement, all the compounds have limited ability to penetrate skin. The Bio-Availability score of all the compounds is 0.55, as it satisfies the rule of five.

3.5 Protein-Ligand Interaction:

The phytocompounds Diosgenone,4-dimethyl-, Tigogenin and Ecliptalbine Showed a good affinity towards the receptor molecule, passed the ADMET and CYP Properties, it depicted that these compounds have the property to be acted as a Lead molecule. The phytocompounds Diosgenone,4-dimethyl-, Tigogenin and Ecliptalbine were visualized using Discovery studio Suite 3.5 and their 2D and 3D interaction plots were obtained. The 2D and 3D interaction plots of the Standard drugs were visualized using Discovery Studio Suite 3.5. **(Fig. 3.5 a – Fig. 3.5 n)**

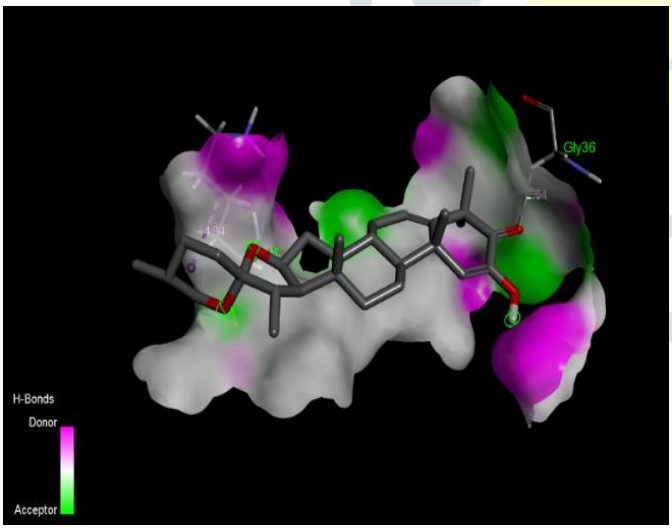

Figure. 3.5 a) 3D interaction of the phytocompound Diosgenone,4-dimethyl- with the target protein.

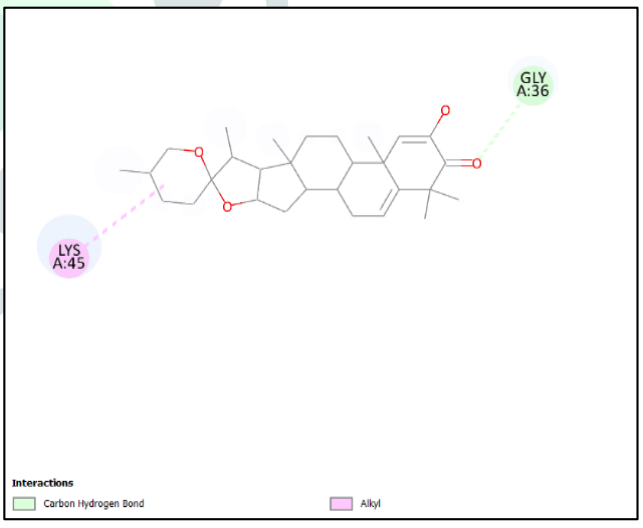

Figure. 3.5 b) 2D interaction of the phytocompound Diosgenone,4-dimethyl- with the target protein.

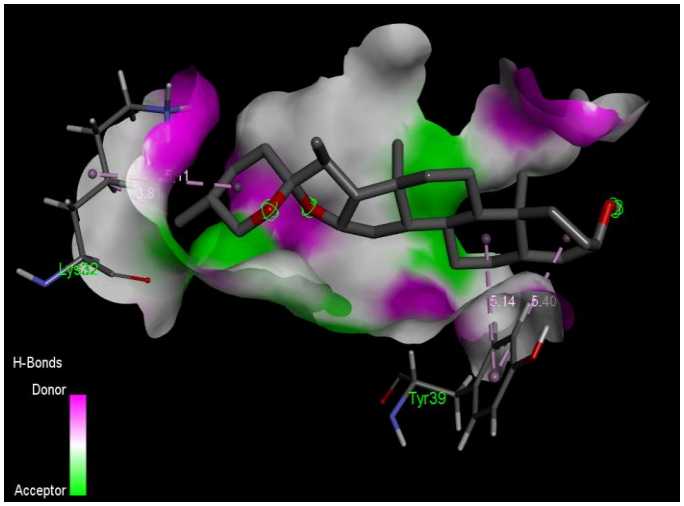

Figure. 3.5 c) 3D interaction of the phytocompound Tigogenin with the target protein.

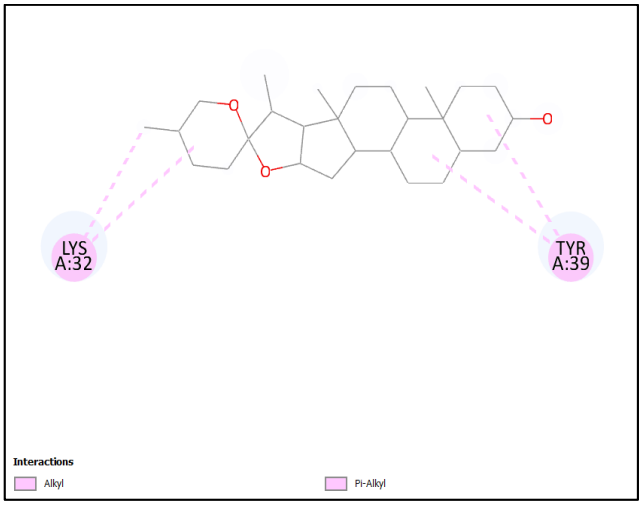

Figure. 3.5 d) 2D interaction of the phytocompound Tigogenin with the target protein.

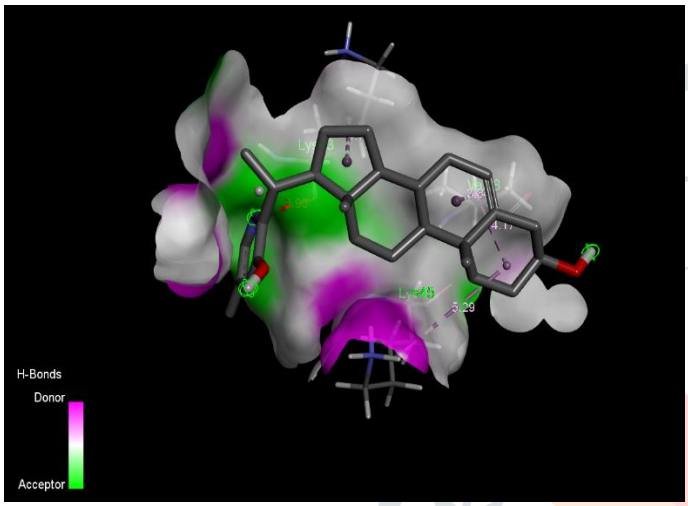

Figure. 3.5 e) 3D interaction of the phytocompound Ecliptalbine with the target protein.

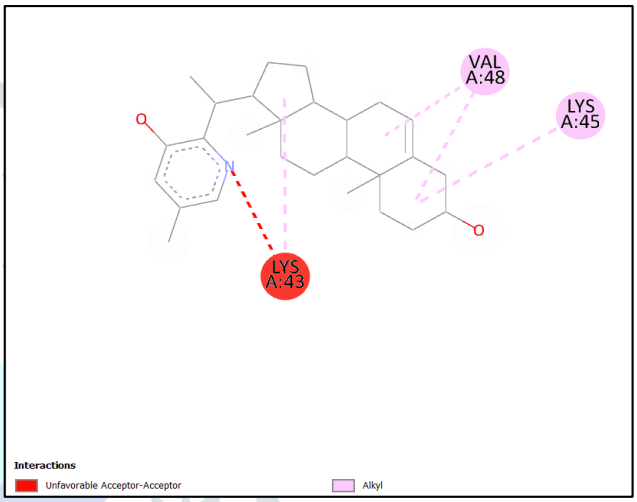

Figure. 3.5 f) 2D interaction of the phytocompound Ecliptalbine with the target protein.

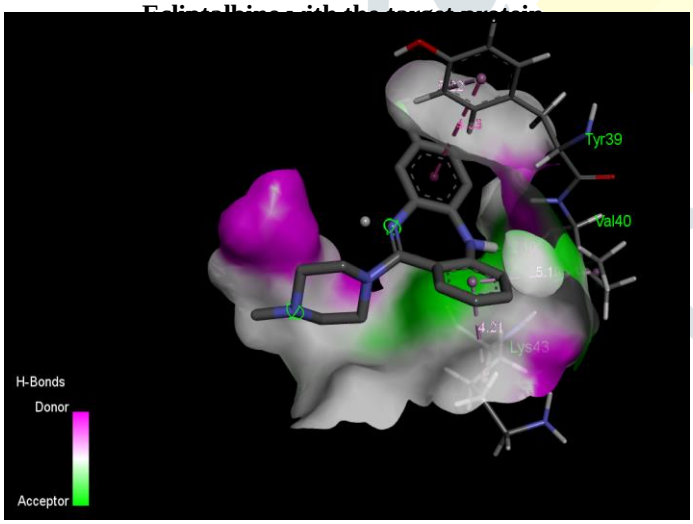

Figure. 3.5 g) 3D interaction of the Synthetic Drug Clozapine with the target protein.

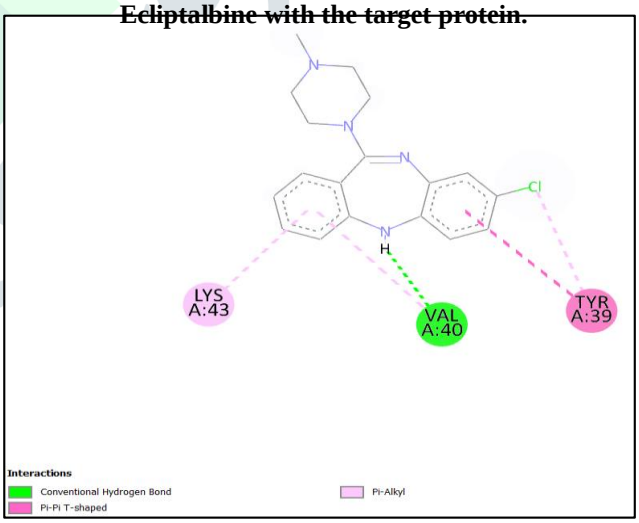

Figure. 3.5 h) 2D interaction of the Synthetic Drug Clozapine with the target protein.

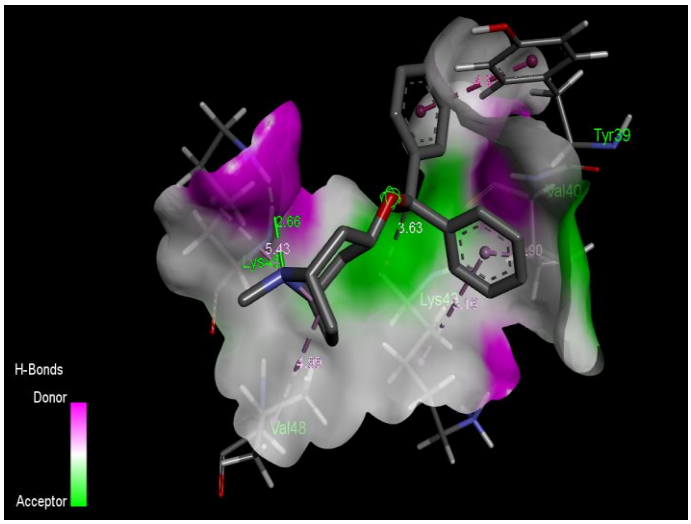

Figure. 3.5 i) 3D interaction of the Synthetic Drug Benztropine with the target protein.

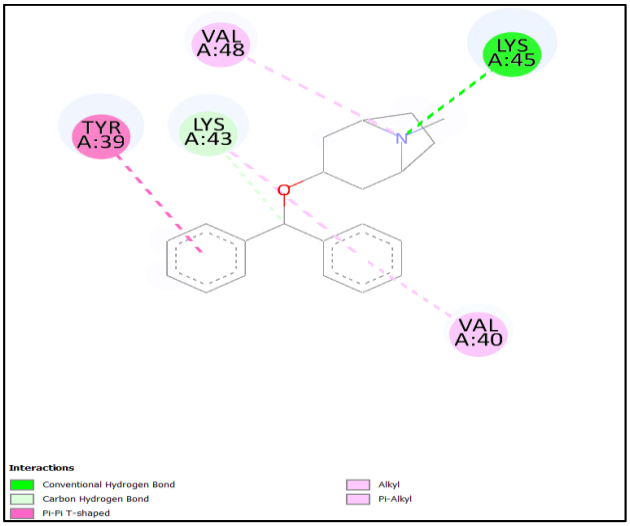

Figure. 3.5 j) 2D interaction of the Synthetic Drug Benztropine with the target protein.

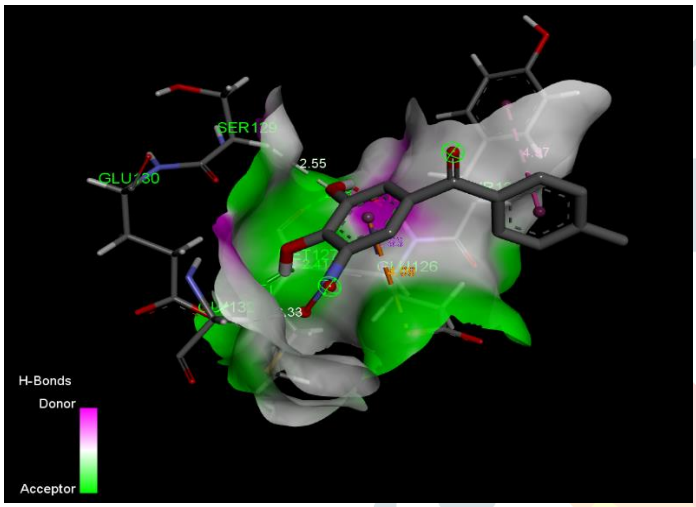

Figure. 3.5 k) 3D interaction of the Synthetic Drug

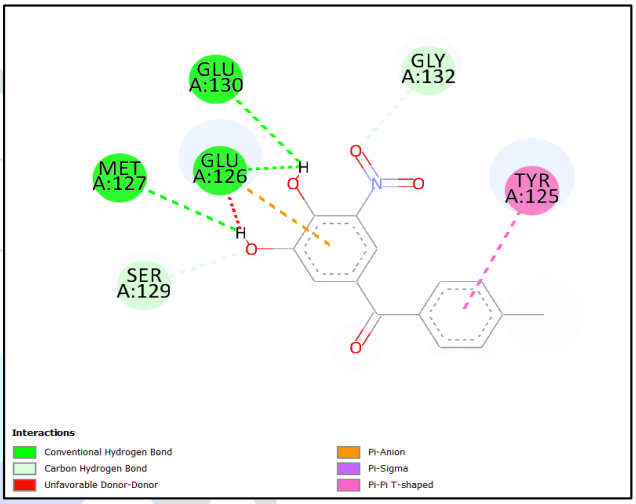

Figure. 3.5 l) 2D interaction of the Synthetic Drug Tolcapone with the target protein.

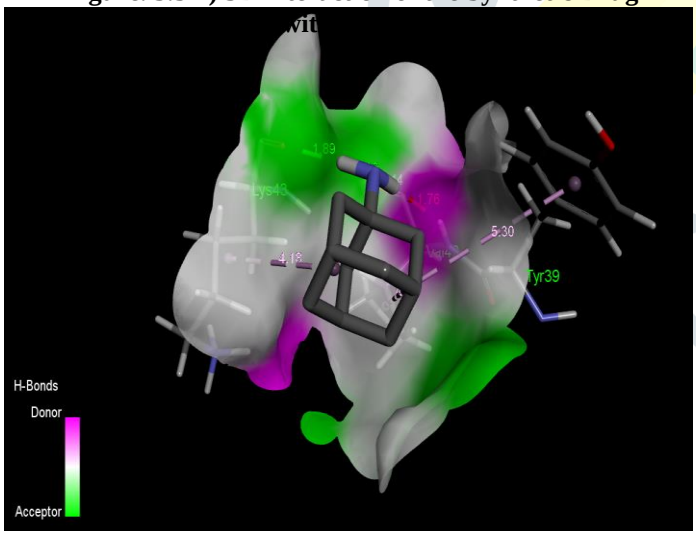

Figure. 3.5 m) 3D interaction of the Synthetic Drug Amantadine with the target protein.

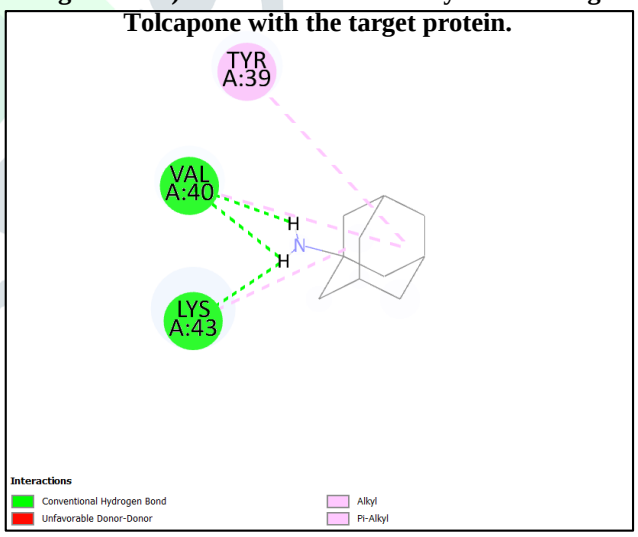

Figure. 3.5 n) 3D interaction of the Synthetic Drug Amantadine with the target protein.

IV. DISCUSSION

According to the study conducted by Elangovan *et al.*, (2013) reported that five plant derived compounds namely Stimovul, 7,8 Dihydroxycoumarin, Etorphine, Estadurin and Pentazdine were taken and docked against Alpha-Synuclein receptor (PDB ID: 1XQ8). The docking scores of these Bioactive compounds against 1XQ8 was -4.5122 Kcal/mol for Stimovul, -4.3881 Kcal/mol for 7,8 dihydroxycoumarin, -3.7081 Kcal/mol for Estadurin, -3.0914 Kcal/mol for Etorphine and for Pentazdine it was -2.9219 Kcal/mol. Based on the results, it was revealed that Stimovul has the ability to prevent the self-aggregation of the protein to form a

toxic aggregate by inhibiting the Alpha-Synuclein receptor. They suggested that Stimovul can be further developed as a potential drug for Parkinson disease. [35]

According to the study conducted by Rajeshkumar *et al.*, BioImpacts, (2022), revealed that the bioactive compounds from the *Cynodon dectylon* can degrade the Alpha-Synuclein in the brain through *in silico* Docking investigations. Twenty-nine bioactive chemicals from *C. dactylon* were used. During Molecular Docking investigation the bioactive compounds Vitexin with -7.3 Kcal/mol and Homoorientin with -7.1 Kcal/mol showed significant binding energy against the target protein. By analyzing overall parameters, it indicates that vitexin can be used as a potential lead compound against α-Syn aggregation. [28]

A study conducted by Thangavel *et al.*, also used *in silico* analysis to find out effective derivatives of 7,8-dihydroxyflavone (DHF) for inhibiting α-Syn aggregation previously. [29]

Alex France M. Monteiro *et al.*, (2018) in his study, he evaluated 39 Flavanoids using prediction of molecular properties and *in silico* docking studies by comparing it against 7 standard reference compounds: 4 for Parkinson's and 3 for Alzheimer's. The docking results for PD using selected flavonoids as compared to the standards with four proteins resulted similar binding energies, indicating that the compounds 8-prenylnaringenin, europinidin, epicatechin gallate, homoeriodictyol, capensinidin, and rosinidin are as potential leads. [5]

The most prevalent reason for the pathological condition of dopaminergic neurons of PD is an atypical aggregation of α-Syn protein in the form of Lewy bodies and Lewy neuritis in neurons [30]. Oxidative stress plays a critical role in the degradation of dopaminergic neurons in PD [31]. Several studies were shown that bioactive compounds from plants have neuroprotective effects against α-Syn aggregation and oxidative stress on PD [32,33]. Hence the primary objective of this study was to find a potential lead compound that can inhibit the receptor of Alpha-Synuclein, which in turn prevents the self-aggregation of lewy bodies that occurs in the midbrain's Snpc.

In silico molecular docking helps in advancing the drug discovery process because the screening of the phytocompounds that bind to the target protein can be predicted in a short duration. This along with *in silico* evaluation of pharmacokinetic, physiochemical, and pharmacodynamics properties supports in the identification of a few lead compounds that can be developed into a drug that can lead to disease prevention [28]. *In silico*-based virtual screening and molecular docking experiments were employed in many studies and the results obtained were later confirmed by in vitro and in vivo activity evaluation studies [34]. This confirms the value of in silico studies in the drug discovery processes. Researchers may be able to find therapeutic solutions for specific diseases by better understanding how the compounds bind to, interact with, and down or upregulate the associated proteins.

The goal of this study was to find a potential lead compound that can inhibit the receptor of Alpha-Synuclein. The phytocompounds from 21 locally available medicinal plants which is known for their Anti-Inflammatory, Anti-Oxidant, Antitumor, Anti-neurotoxic, Neuroprotective activity etc., which could be used to treat Parkinson's Disease are taken. Using IMPPAT Database 1087 phytocompounds were retrieved. Lipinski Rule of Five was assessed for the retrieved compounds and among those 854 compounds exhibited Drug like property. The compounds were further taken into Molecular docking studies using PyRx V0.8 software and the docking scores of the chosen phytocompounds against Alpha-Synuclein receptor (PDB ID: 1XQ8) range from -2.8 Kcal/mol to -8.3 Kcal/mol. Among them, top 10 compounds were taken into recurrent study and compared with 4 standard drugs Clozapine, Tolcapone, Benztropine and Amantadine which are used to treat Parkinson's Disease. The top 10 compounds and 4 Synthetic drugs were assessed with the ADMET and CYP properties using SwissADME. The Boiled Egg parameter was used to predict the Blood-Brain Barrier permeation and P-Glycoprotein substrate of the phytocompounds and Standard drugs. While analyzing the results, the phytocompounds Diosgenone,4-dimethyl, Tigogenin and Ecliptalbine were no +PGP Substrate, had a High Intestinal Absorption (HIA) and passed every pharmacokinetic and CYP properties. In these three compounds, Tigogenin from ***Cheilocostus speciosus*** had permeated through the Blood-Brain Barrier and hence it will be a suitable drug candidate which can act as a Lead compound by inhibiting the active site of the Alpha-Synuclein receptor in order to prevent the self-association of them into Lewy bodies.

V. CONCLUSION

In the present study, 1087 phytocompounds from 21 locally available medicinal plants and the target protein Alpha-Synuclein (PDB ID: 1XQ8) were subjected to Docking analysis to find the potential inhibitors for Alpha-Synuclein receptor in order to prevent the self-association of them into lewy bodies. In which, 854 phytocompounds passed Lipinski Rule of Five and according to the binding affinity, top 10 phytocompounds were taken for further analysis. The Top 10 phytocompounds were compared with the 4 standard drugs Clozapine, Tolcapone, Benztropine and Amantadine which are used in the treatment of Parkinson's Disease. Those phytocompounds showed better results than the standard drugs. Toxicity analysis was done for the 10 best interacted phytocompounds and the results concluded that the compounds had very less toxicity. The phytocompound Diosgenone,4-dimethyl, Tigogenin and Ecliptalbine with binding affinity -7.9 Kcal/mol, -7.6 Kcal/mol and -7.4 Kcal/mol respectively, had good interaction with the target protein. Based on the ADMET and CYP Properties, the phytocompounds Diosgenone,4-dimethyl, Tigogenin and Ecliptalbine were no +PGP Substrate, had a High Intestinal Absorption (HIA), passed every pharmacokinetic property and additionally, Tigogenin from *Cheilocostus speciosus* had permeated through the Blood-Brain Barrier. Hence, the present study concludes that Tigogenin from *Cheilocostus speciosus* can be used as a Lead compound in the treatment of Parkinson's Disease.

VI. ACKNOWLEDGMENT

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