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In-Silico Study of – 39 Phytochemicals Targeted Against Micelle-Bound and Parkinson's Diseased Patient's 'A-Synuclein'

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ABSTRACT

'α-Synuclein' is encoded by SNCA gene, and is made up of 140 amino acids. The mutations Glu46Lys, His50Gln, and Ala53Glu can lead to the formation of insoluble aggregates and oligomers of α-Synuclein. Loss of dopaminergic neurons in the substantia nigra pars compacta and aggregation of 'α-Synuclein' protein in the form of Lewy bodies or Lewy neurites are the neuropathological hallmarks of Parkinson's disease. This 'α-Synuclein' can spread from one cell to another, and also, from one region to other region, which dramatically promotes pathogenesis and progression of Parkinson's disease. Curcumin has shown to prevent this aggregation of alpha-synuclein oligomer. In this research we made an attempt to find more phytochemicals like 'Curcumin' which can serve as the alternatives of 'Curcumin' against 'α-Synuclein'. This in-silico research involved the docking analysis of 'Curcumin' and other 39 different phytochemicals against 2 different structures of α-Synuclein having PDB IDs – '1XQ8' and '7XO1'. For this purpose 39 different phytochemicals were collected from IMPPAT database. Docking analysis showed that, Mahanimbine', 'Ergosterol' and 'Laurotetanine' bound to the same cavity size of Alpha-Synuclein to which 'Curcumin' bound, and, these showed more negative vina scores than 'Curcumin'. The binding affinity of the 2 different structures of α-Synuclein also varied. The binding affinity for most of these phytochemicals was found to reduce in the α-Synuclein of PD patients.

Keywords: α-Synuclein, Curcumin, Docking, Binding affinity

INTRODUCTION

Neurodegeneration is the irreversible process of loss of neurons. This loss of neurons, leads to the continuous loss of the structural and the functional aspects of the degenerating neurons. **[1].** It is characterized by progressive neuronal death, which may lead to the development of cognitive or motor symptoms, in the later stages, depending upon, the area of the brain where this neurodegeneration is occurring. **[2].** Few commonly known examples of the diseases, caused by neurodegeneration are – Parkinson's disease, Alzheimer's disease, Huntington's disease, etc. **[1].** Aging, which leads to the progressive deterioration of the normal biological functions of our body, is a major risk factor responsible for neurodegenerative diseases. Oxidative stress and nitrosative stress are the common processes involved in, many of the neurodegenerative diseases. According to free radical theory of aging, when 'reactive oxygen species (ROS)' and 'reactive nitrogen species (RNS)' rises, neural membranes get damaged and oxidative and nitrosative stress gets induced. These two stresses, can lead

to the decline in the cognitive and motor performances, in the elderly population, even if neurodegenerative diseases are absent in them. Although, high rates of these two types of stress, are the major factors, which may have some role in the pathogenesis of neurodegenerative diseases. Diet is another environmental factor, which may have role in preventing these neurodegenerative diseases. If our diet is supplemented with – polyphenols, resveratrol, ginkgo biloba, curcumin, ferulic acid, carotenoids, flavonoids, and n-3 fatty acids, then they maybe beneficial to us in multiple ways. They may help in scavenging free radicals, and, may also help in modulating signal transduction, gene expression, and restoring optimal neuronal communication. **[3].** The central cause of neurodegeneration are the genetic factors. Genetics may also play an important role in the translational research, which can aim to develop the novel disease-modifying therapies for neurodegenerative disorders. **[4].**

Previous investigations suggests that, the survival rates of the patients of neurodegenerative diseases are limited. In 2019, throughout the world, 10 million deaths occurred and 349.2 million people were effected by the major neurological disorders. **[5,6,7].** In the same year, which is 2019, the number of patients of Parkinson's disease, increased beyond 8.5 million. Some recent estimates, also suggest that Parkinson's disease led to 5.8 million disability-adjusted lives. This showed the 81% surge of PD since 2,000. **[7,8].** Moreover, Parkinson's disease was also responsible for 3,29,000 deaths. This was also marked as an increase of the number of patients of this disease since 2,000. **[7].**

Parkinson's disease is the second most common progressive neurodegenerative disorders. **[9,10].** In this disease, the dopaminergic neurons degenerate in the substantia nigra of the midbrain. The symptoms of this disease, which develop as a result of this neurodegeneration, can be categorized into motor and non-motor symptoms. Classically, the patients of this disease show rest tremor, rigidity, bradykinesia, and, stooping posture. However, this disease can also be associated with neurobehavioral disorders, such as depression, anxiety, cognitive impairment such as dementia, and, autonomic dysfunction. **[9].** The main neuropathology of this disease, is the cell (neurons) death in the substantia nigra, due to the deposition of lewy bodies. Because of this, dopamine denervation occurs. But, so many extra-nigral and non-dopaminergic regions of our brain are also affected by this disease. **[10].** This disease is diagnosed clinically and requires bradykinesia. **Bradykinesia** is the 'slowness of movement or decrease of amplitude or speed', which is usually assessed using finger tapping, foot tapping or pronation-supination hand movements. **[10, 11].** Additionally, rest tremor or rigidity is also required to confirm a parkinsonian syndrome. However tremor was found to be absent in 30% of confirmed PD cases. **[10, 12].**

Currently, there is no such confirmed treatment of Parkinson's disease, which exhibit disease-modifying or neuroprotective effect. **[10,13].** Currently available evidence-based confirmed medicines are given, for the treatment of symptoms, and, they mainly work upon dopaminergic replacement or modulation. Levodopa, dopamine agonists and Monoamine oxidase B inhibitors are all licensed to given to the patients of Parkinson's disease. Anticholinergics are not used routinely, due to the risk of cognitive decompensation. **[10,14].** 'Levodopa' may be given in combination with 'Carbidopa' to treat the symptoms of Parkinson's disease. Some of the dopamine agonists, which can be given to the patients are – Pramipexole, Ropinirole, Rotigotine etc. For the inhibition of Monoamine oxidase enzyme, to reduce central dopamine catabolism, Selegiline, Rasagiline, etc. maybe given to the patients. Other than these, Entacapone, Opicapone and Tolcapone, maybe given as COMT inhibitor, and, Amantadine maybe given as NMDAR antagonist, to the patients of Parkinson's disease. **[10].**

'α-Synuclein' is encoded by SNCA gene. It is made up of 140 amino acids. The mutations associated with Parkinson's disease are Ala53Thr, Ala30Pro, Glu46Lys, His50Gln, Gly51Asp, and Ala53Glu, which are found in N-terminal domain. **[25].** The mutations Glu46Lys, His50Gln, and Ala53Glu can lead to the formation of insoluble aggregates and oligomers of α-Synuclein. **[26, 27, 28].** When 'α-Synuclein' is phosphorylated it is necessary and sufficient in the process of degradation in neurodegenerative diseases. Alpha synuclein which was isolated from human Lewy bodies was phosphorylated at S129. **[29].** Loss of dopaminergic neurons in the substantia nigra pars compacta and aggregation of 'α-Synuclein' protein in the form of Lewy bodies or Lewy neurites are the neuropathological hallmarks of Parkinson's disease. **[37].** Many *in-vitro* and *in-vivo* studies suggest that, 'α-Synuclein' can spread from one cell to another, and also, from one region to other region, which dramatically promotes pathogenesis and progression of Parkinson's disease. **[30-37].**

'Curcumin' is an active ingredient in the turmeric. Curcumin is lipophilic in nature, therefore, it can cross the blood-brain barrier. **[38, 39].** It can exhibit, different protective activities in our brain, such as, protection against toxic metals and reactive oxygen species. Curcumin is a flavonoid, whose anti-oxidant properties are stronger than other antioxidants like Vitamin C and E. **[38, 40].** It donates H ion to protect mitochondria and neurons from getting damaged by the reactive oxygen species. In Parkinson's disease, oligomers of alpha-synuclein aggregates to form Lewy bodies. Curcumin has shown to prevent this aggregation of alpha-synuclein oligomer. **[38, 41].**

In this research, we made an attempt to find more phytochemicals like 'Curcumin' which can bind to 'Alpha-Synuclein'. To achieve this attempt, 39 phytochemicals have been targeted in-silico in this research, against the two different structures of Alpha-Synuclein having PDB Ids '1XQ8' and '7XO1'.

MATERIALS and METHODS

Tools and Databases:

1. **IMPPAT 2.0 database: '**IMPPAT 2.0' is an enhanced and expanded database. It has information on 4,010 Indian medicinal plants, 17,967 phytochemicals, and, 1095 therapeutic uses. It contains the information about the associations at the level of plant parts. Overall, it is a manually curated database, which contains phytochemical atlas of Indian medicinal plants. **[15].** In this research, this database have been used for the collection of phytochemicals, to be utilized as ligands.
2. **Uniprot database:** Uniprot provides comprehensive, high quality and free to access set of protein sequences, annotated with the information about their functions. The number of sequences in UniprotKB have increased up to approximately 190 million till 2021.**[16].** This database is actually a collection of sequences of proteins and their annotations. Many of the sequences are coming from genome sequencing projects. **[17].** In this research, this database has been used to study about 'α-synuclein'.
3. **Protein Data Bank (RCSB-PDB):** It stands for 'Research Collaboratory for Structural Bioinformatics Protein Data Bank.' **[18, 20].** It is an open access database of Biology which shares the 3-Dimentional structures of proteins. **[19, 20].** It was established in 1971 with 7 structures. But, over the period of time it became possible to access more than 113,000 entries of natural and designed macromolecules. More than 84,000 of those macromolecules are complexed with small chemical components, such as solvent, ions, cofactors, inhibitors and drugs. This database was originally designed for the structural biology community, but, over the period of time, other

professionals also began to use it. **[20].** In this research, this database have been used to visualize and obtain the 3-Dimentional structures of micelle-bound and PD's Alpha-synuclein.

4. **https://project-gemmi.github.io/wasm/convert/cif2pdb.html :** In this research, this link was used to convert .cif files to .pdb files.
5. **BIOVIA Discovery studio:** This tool is mainly used for protein cleaning. However, in this research the two structures of α-synuclein didn't require cleaning at all.
6. **PubChem:** It is a public repository, which provides information about various chemical substances. It is a database of NCBI. It was launched in 2004. It is a component of Molecular Libraries Roadmap Initiatives of US National Institute of Health. Since years, this database has been serving as a resource for chemical information for the scientific research community. **[21].** In this research, PubChem has been used to study the chemistry of ligands, and, download the 3-Dimentional structures of the ligands in '.sdf ' format.
7. **CACTUS Online SMILES Translator:** It belongs to NCI/CADD group, NIH-National Cancer Institute. **[22].** In this research, this tool has been used to convert the .sdf files of ligands into .pdb files, so that those 3D structures of ligands can be used for docking.
8. **CB-Dock:** It is a user-friendly web server for blind docking. It predict binding modes without information about binding sites. It predicts binding sites of a given protein and calculates the centres and sizes with a new curvature-based cavity detection approach. It performs docking with 'Autodock Vina'. It provides an interactive 3-Dimentional visualization of results, and is available free of cost at http://clab.labshare.cn/cb-dock/. **[23].** In this research, this tool has been used for the blind docking of 39 phytochemicals against the two different structures of 'Alpha-Synuclein'.
9. **pkCSM tool:** It is a novel approach for the prediction of ADMET properties. It uses graph-based signatures to develop the predictive models of central ADMET properties. **[24].** In this research, this tool has been used to analyse the Lipinski's rule of 5 and different parameters related to Absorption, Distribution, Metabolism, Excretion and Toxicity.

Methodology:

1. Target protein identification and computation of docking score of 'Curcumin':

This step began with the literature surveys. Through literature survey, we got to know that, aggregation of Alpha-Synuclein, leading to the formation of Lewy bodies, is the hallmark of Parkinson's disease. Through more literature survey, we also got to know that, 'Curcumin' has been found, to prevent the aggregation of Alpha-Synuclein oligomers. We wanted to explore more phytochemicals like 'Curcumin', as its alternatives against Alpha-Synuclein. Thus, we decided to target the micelle-bound Alpha-Synuclein and the Alpha-Synuclein isolated from the CSF of the patient of Parkinson's disease, because we also wanted to analyse the variations in the binding affinity of the Alpha-Synuclein of a healthy person and Parkinson's diseased patients. Thus, we searched about the Alpha-Synuclein protein having Uniprot ID 'P37840'. Then, we chose the proteins having PDB IDs '1XQ8' and '7XO1' and downloaded their structures in '.cif' format. Then, '.cif' files were coverted into '.pdb' format using https://project-gemmi.github.io/wasm/convert/cif2pdb.html. These 2 proteins didn't require cleaning using Discovery Studio, as these were downloaded in cleaned form itself. After that, 3-Dimentional structure of 'Curcumin' was downloaded from 'PubChem' in '.sdf' format. Then, it was converted into '.pdb' format using 'CACTUS Online SMILES Translator'. Then, 'Curcumin' was docked against the two different structures of Alpha-Synuclein, having PDB Ids - '1XQ8' and '7XO1', using 'CB-Dock'.

On analysis by CB-Dock, 'Curcumin' has shown the minimum Vina score of '-6' against the 'Micelle-bound alpha-synuclein found in *Homo sapiens'* in its cavity size of '285', whereas, it has shown the minimum Vina score of '-5.2' against the 'Alpha-Synuclein obtained from the CSF of the Parkinson's diseased patient' in its cavity size of '145'. **[46].**

2. Collection of ligands:

To explore more phytochemicals like 'Curcumin' against 'Alpha-Synuclein', we collected 39 different phytochemicals from 'IMPPAT 2.0 database' from different plant sources. Many of these plant sources and phytochemicals have been found to be effective on our brain. Thus, these phytochemicals were chosen as target ligands against 'Alpha-Synuclein' also, as the aim to target Parkinson's disease.

3. Docking:

This step involved the blind docking of 39 ligands, against the two different structures of, 'Alpha-Synuclein', using 'CB-Dock'. Their vina scores and binding locations were analysed using this tool.

4. Lipinski's rule of 5 analysis:

It is a rule of thumb which tells us whether a chemical compound with specific pharmacological properties can be an orally active drug in humans or not. This rule was based on the observation that the effective drugs which are orally consumed are small and lipophilic. This rule of 5 tells us that a drug is likely to be poorly absorbed, if –

- It has more than 5 hydrogen bond donors,
- It has more than 10 (5*2) hydrogen bond acceptors,
- It's molecular weight is greater than 500,
- A calculated Log P greater than 5. **[42].**
- No. of rotatable bonds are more than 10.

In this research, the above-mentioned parameters have been analysed for 'Curcumin', 'Mahanimbine', 'Ergosterol' and 'Laurotetanine' using the pkCSM tool. These phytochemicals were preferred for further analysis, because, these gave better 'vina scores' against 'Alpha-Synuclein', as compared to 'Curcumin', in the same cavity sizes to which 'Curcumin' bound.

5. ADMET analysis of phytochemicals:

In this step, 'Curcumin', 'Mahanimbine', 'Ergosterol' and 'Laurotetanine' have been analysed for their Absorption, Distribution, Metabolism, Excreation and Toxicity related parameters using pkCSM tool.

RESULTS

1. Target proteins:

Figure-1.1: ***3-Dimentional structure of Micelle-bound alpha-synuclein found in Homo sapiens (Human beings).*** **[43].**

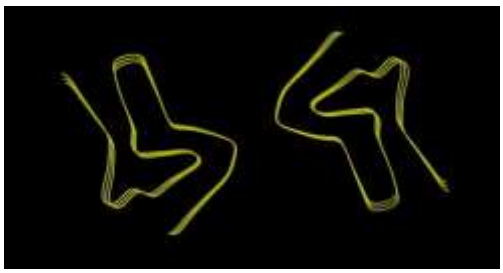


Figure-1.2: ***3-Dimentional structure of major polymorph in alpha-synuclein fibril seeded by Cerebrospinal Fluid from a patient with mid-to-late stage Parkinson's disease.*** **[44].**

2. Collection of ligands:

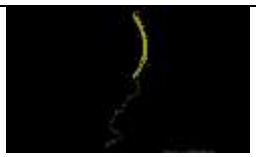
S.No.	***Phytochemicals (Ligands)***	***Medicinal plants***
1.	*Catharanthine*	*Catharanthusroseus(Madagascar Periwinkle)*
2.	*Cryptolepine*	*Cryptolepis Sanguinolena (nibima, kadze, gangamau, Ghanaian quinine and yellow-dye root)*
3.	*Mahanimbine*	*Murrayakoenigii (Curry Leaf Tree)*
4.	*Friedelin*	*Calophyllumpinetorum, .*
5.	*Lycopsamine*	*Ageratum conyzoides (Billygoat-weed)*
6.	*Myristicacid*	*Abelmoschusesculentus (Ladies finger, Okra - Seeds)*
7.	*Malvalic acid*	*Abelmoschusesculentus (Ladies finger, Okra - Seeds)*
8.	*Pentadecanal*	*Abelmoschusesculentus (Ladies finger, Okra - Seeds)*
9.	*Methyl linoleate*	*Abelmoschusesculentus (Ladies finger, Okra - Seeds)*
10.	*Ethyl octanoate*	*Abelmoschusesculentus (Ladies finger, Okra - Seeds)*
11.	*Sterculicacid*	*Abelmoschusesculentus (Ladies finger, Okra - Seeds)*
12.	*Ethyl tetradecanoate*	*Abelmoschusesculentus (Ladies finger, Okra - Seeds)*
13.	*Ergosterol*	*Abelmoschusmoschatus (Musk mallow)*
14.	*Stigmasterol*	*Abelmoschusmoschatus (Musk mallow)*
15.	*Laurotetanine*	*Actinodaphneobovata*

16.	Heliotridine	Ageratum houstonianm (flossflower)
17.	Retronecine	Ageratum houstonianm (flossflower)
18.	1-Methoxycanthinone	Ailanthus altissima (Tree of heaven)
19.	Tropone	Acoruscalamus (Sweet flag)
20.	Eucalyptol	Abutilon indicum
21.	Apiole	Acacia caven
22.	Leucoxol	Acacia leucophloea
23.	Beta-Cubebene	Abelmoschusesculentus
24.	Santene	Abies alba
25.	Camphene	Abies alba
26.	Beta-Bisabolene	Ocimum tenuiflorum (Leaf)
27.	Samaderine E	Ocimum tenuiflorum (Leaf)
28.	Isophytol	Ocimum tenuiflorum (Aerial part)
29.	Furfural	Ocimum tenuiflorum (Aerial part)
30.	beta-Elemene	Ocimum tenuiflorum (Aerial part)
31.	Cubebol	Abies alba (Stem)
32.	Juniperol	Abies alba (Stem)
33.	Cyclofenchene	Cupressus sempervirens (Leaf)
34.	Sabinene	Abies alba (Stem and Leaf)
35.	Beyerene	Hyptis suaveolens (Leaf)
36.	3-Carene	Abies pindrow (Leaf)
37.	D-Glucoronic acid	Acacia catechu (Plant exudate)

38.	*D-Rhamnose*	*Acacia catechu (Bark)*
39.	*L-Arabinose*	*Acacia catechu (Bark)*

Table-1: List of phytochemicals collected as ligands from IMPPAT database. [45].

3. Docking analysis of phytochemicals:

Protein	Micelle-bound alpha-synuclein found in *Homo sapiens* (Human beings)		Major polymorph in alpha-synuclein fibril seeded by Cerebrospinal Fluid from a patient with mid-to-late stage Parkinson's disease.	
PDB ID	1XQ8		7XO1	
Structure of protein				
Phytochemicals	**Minimum vina score**	**Cavity size**	**Minimum vina score**	**Cavity size**
Catharanthine	-6.5	164	-5.3	83
Cryptolepine	-6	164	-5.2	82
Mahanimbine	-6.7	285	-6.6	83
Friedelin	-7.3	164	-6.4	83
Lycopsamine	-5	164	-5	83
Myristic acid	-4	164	-3.6	83
Malvalic acid	-4.6	164	-4.3	82
Pentadecanal	-3.4	164	-3.5	83
Methyl linoleate	-3.7	164	-3.8	83
Ethyl octanoate	-3.9	164	-3.6	83
Sterculic acid	-4.1	164	-4	115
Ethyl tetradecanoate	-3.6	164	-3.5	145
Ergosterol	-6.7	164	-6.4	145
Stigmasterol	-6.9	164	-6.6	83
Laurotetanine	-5.9	164	-5.6	145
Heliotridine	-4.6	285	-3.7	145
Retronecine	-4.2	285	-3.5	83
1-Methoxycanthinone	-5.7	164	-5.1	83
Tropone	-3.8	164	-3.1	83
Eucalyptol	-4.3	164	-2.7	83
Apiole	-4.6	164	-3.9	83
Leucoxol	-6.6	164	-5.6	82

Beta-Cubebene	-5.5	164	-4.7	82
Santene	-3.9	164	-3.2	83
Camphene	-4.1	164	-2.7	83
Beta-Bisabolene	-4.6	164	-4.9	83
Samaderine E	-6.7	164	-5.8	83
Isophytol	-4.1	164	-4.3	83
Furfural	-3.1	164	-3.1	83
Beta-Elemene	-4.7	164	-4.2	82
Cubebol	-5.3	164	-4.6	145
Juniperol	-5.1	164	-3	145
Cyclofenchene	-4.2	164	-2.5	83
Sabinene	-4.1	164	-3.3	82
Beyerene	-6.1	164	-5.8	83
3-Carene	-4.2	164	-3.6	82
D-Glucoronic acid	-4.4	285	-3.9	115
D-Rahmnose	-4.1	164	-4.3	83
L-Arabinose	-4	285	-3.7	83

Table-2: CB-dock analysis of 39 phytochemicals against 2 different structures of Alpha-Synuclein. [46].

4. Lipinski's rule of 5 analysis:

ADME Property	Value	No violation (As per Lipinski's rule of 5.)
Molecular weight	368.385	No violation
No. of hydrogen bond donor	2	No violation
No. of hydrogen bond acceptor	6	No violation
LogP	3.3699	No violation
No. of rotatable bonds	8	No violation

Table-3.1: Lipinski's rule of 5 analysis of 'Curcumin' (pkCSM) [47].

ADME Property	Value	No violation (As per Lipinski's rule of 5.)
Molecular weight	331.459	No violation
No. of hydrogen bond donor	1	No violation
No. of hydrogen bond acceptor	1	No violation
LogP	6.54022	Violation
No. of rotatable bonds	3	No violation

Table-3.2: Lipinski's rule of 5 analysis of 'Mahanimbine' (pkCSM) [47].

ADME Property	Value	No violation (As per Lipinski's rule of 5.)
Molecular weight	396.659	No violation

No. of hydrogen bond donor	1	No violation
No. of hydrogen bond acceptor	1	No violation
LogP	7.3308	Violation
No. of rotatable bonds	4	No violation

Table-3.3: Lipinski's rule of 5 analysis of 'Ergosterol' (pkCSM) [47].

ADME Property	**Value**	**No violation (As per Lipinski's rule of 5.)**
Molecular weight	327.38	No violation
No. of hydrogen bond donor	2	No violation
No. of hydrogen bond acceptor	5	No violation
LogP	2.8279	No violation
No. of rotatable bonds	3	No violation

Table-3.4: Lipinski's rule of 5 analysis of 'Laurotetanine' (pkCSM) [47].

5. ADMET analysis:

ADMET property	**Model name**	**Curcumin**	**Mahanim-bine**	**Ergoster-ol**	**Laurotetan-ine**
Absorption	Water solubility	-4.01	-6.2	-6.696	-2.981
Absorption	CaCO2 permeability	-0.093	1.046	1.218	1.21
Absorption	Intestinal absorption (Human)	82.19	91.746	95.41	94.327
Absorption	Skin permeability	-2.764	-2.488	-2.811	-2.82
Absorption	P-glycoprotein substrate	Yes	Yes	No	Yes
Absorption	P-glycoprotein I inhibitor	Yes	Yes	Yes	No
Absorption	P-glycoprotein II inhibitor	Yes	Yes	Yes	Yes
Distribution	VDss (Human)	-0.215	1.063	0.272	1.367
Distribution	Fraction unbound (Human)	0	0	0	0.303
Distribution	BBB permeability	-0.562	0.28	0.764	-0.257
Distribution	CNS permeability	-2.99	-1.647	-1.752	-2.313
Metabolism	CYP2D6 substrate	No	No	No	No
Metabolism	CYP3A4 substrate	Yes	Yes	Yes	Yes
Metabolism	CYP1A2 inhibitor	Yes	Yes	No	Yes
Metabolism	CYP2C19 inhibitor	Yes	Yes	No	No
Metabolism	CYP2C9 inhibitor	Yes	No	No	No
Metabolism	CYP2D6 inhibitor	No	No	No	Yes

Metabolism	CYP3A4 inhibitor	Yes	No	No	No
Excretion	Total clearance	-0.002	0.556	0.564	1.019
Excretion	Renal OCT2 substrate	No	No	No	No
Toxicity	AMES toxicity	No	Yes	No	Yes
Toxicity	Max tolerated dose (human)	0.081	0.239	-0.691	-0.061
Toxicity	hERG I inhibitor	No	No	No	No
Toxicity	hERG II inhibitor	No	Yes	Yes	Yes
Toxicity	Oral Rat Acute Toxicity (LD50)	1.833	3.304	2.255	3.348
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	2.228	0.799	0.833	0.77
Toxicity	Hepatotoxicity	No	Yes	No	No
Toxicity	Skin sensitisation	No	No	No	No
Toxicity	*T.Pyriformis* toxicity	0.494	0.947	0.517	0.291
Toxicity	Minnow toxicity	-0.081	-0.754	-1.637	1.531

Table-4: ADMET analysis of Curcumin, Mahanimbine, Ergosterol and Laurotetanine from pkCSM tool. [47].

DISCUSSION

Parkinson's disease is the second most common progressive neurodegenerative disorders. In this disease, the dopaminergic neurons degenerate in the substantia nigra of the midbrain. The symptoms of this disease, which develop as a result of this neurodegeneration, can be categorized into motor and non-motor symptoms. Classically, the patients of this disease show rest tremor, rigidity, bradykinesia, and, stooping posture. However, this disease can also be associated with neurobehavioral disorders, such as depression, anxiety, cognitive impairment such as dementia, and, autonomic dysfunction. Loss of dopaminergic neurons in the substantia nigra pars compacta and aggregation of 'α-Synuclein' protein in the form of Lewy bodies or Lewy neurites are the neuropathological hallmarks of Parkinson's disease. The mutations Glu46Lys, His50Gln, and Ala53Glu can lead to the formation of insoluble aggregates and oligomers of α-Synuclein. Many *in-vitro* and *in-vivo* studies suggest that, 'α-Synuclein' can spread from one cell to another, and also, from one region to other region, which dramatically promotes pathogenesis and progression of Parkinson's disease. **'**Curcumin' is an active ingredient in the turmeric. Curcumin is lipophilic in nature, therefore, it can cross the blood-brain barrier. In Parkinson's disease, oligomers of alpha-synuclein aggregates to form Lewy bodies. Curcumin has shown to prevent this aggregation of alpha-synuclein oligomer. Therefore, in this research we've made an attempt to search for more such phytochemicals, which can be the alternatives of 'Curcumin' against 'α-Synuclein'. We targeted the micelle-bound Alpha-Synuclein and the Alpha-Synuclein isolated from the CSF of the patient of Parkinson's disease, because we also wanted to analyse the variations in the binding affinity of the Alpha-Synuclein of a healthy person and Parkinson's diseased patients. Thus, we searched about the Alpha-Synuclein protein having Uniprot ID 'P37840'. Then, we chose the proteins having PDB IDs '1XQ8' and '7XO1' and downloaded their structures in '.cif' format. Then, '.cif' files were coverted

into '.pdb' format using https://project-gemmi.github.io/wasm/convert/cif2pdb.html. These 2 proteins didn't require cleaning using Discovery Studio, as these were downloaded in cleaned form itself. After that, 3-Dimentional structure of 'Curcumin' was downloaded from 'PubChem' in '.sdf' format. Then, it was converted into '.pdb' format using 'CACTUS Online SMILES Translator'. Then, 'Curcumin' was docked against the two different structures of Alpha-Synuclein, having PDB Ids - '1XQ8' and '7XO1', using 'CB-Dock'. On analysis by CB-Dock, 'Curcumin' has shown the minimum Vina score of '-6' against the 'Micelle-bound alpha-synuclein found in *Homo sapiens'* in its cavity size of '285', whereas, it has shown the minimum Vina score of '-5.2' against the 'Alpha-Synuclein obtained from the CSF of the Parkinson's diseased patient' in its cavity size of '145'. To explore more phytochemicals like 'Curcumin' against 'Alpha-Synuclein', we collected 39 different phytochemicals from 'IMPPAT 2.0 database' from different plant sources. Then we performed the blind docking of these 39 ligands, against the two different structures of, 'Alpha-Synuclein', using 'CB-Dock'. We got to know that, 'Mahanimbine', 'Ergosterol' and 'Laurotetanine' are binding to the same cavity size of 'Alpha-Synuclein' to which 'Curcumin' bound, and, their vina scores were also more negative as compared to 'Curcumin'. Therefore these phytochemicals along with Curcumin were selected for the analysis of Lipinski's rule of 5 and different parameters of Absorption, Distribution, Metabolism, Excreation and Toxicity were also analysed for them. This research, mainly showed that, the binding affinity of 'α-Synuclein' varies between the healthy people and the patients having Parkinson's disease, and, the results of CB-Dock analysis of this research, showed that, 'Mahanimbine', 'Ergosterol' and 'Laurotetanine' can be alternatives of 'Curcumin' against 'α-Synuclein'. However, 'Mahanimbine' and 'Ergosterol' was found to be somewhat violating from the 'Lipinski's rule of 5' in terms of 'LogP'. From this in-silico research, we can also conclude that, "The binding affinity of 'Alpha-synuclein' mostly reduces for many of the phytochemicals, when its structure changes in Parkinson's disease. However, in some cases, this binding affinity against the phytochemicals, may remain almost the same or may also get slightly increased, when Alpha-synuclein's structural changes happens in the patients of Parkinson's disease."

CONCLUSION

1. On the basis of the minimum vina scores obtained from 'CB-Dock' analysis, it can be said that, **"The binding affinity of 'Alpha-synuclein' mostly reduces for many of the phytochemicals, when its structure changes in Parkinson's disease. However, in some cases, this binding affinity against the phytochemicals, may remain almost the same or may also get slightly increased, when Alpha-synuclein's structural changes happens in the patients of Parkinson's disease."** – This is, '**Ayushi's in-silico theory of Alpha-Synuclein's binding affinity changes in Parkinson's disease.'**
2. On the basis of analysis using CB-Dock, it can be said that, 'Mahanimbine', 'Ergosterol' and 'Laurotetanine' can be alternatives of 'Curcumin' against 'Alpha-Synuclein' as they bound to the same cavity size to which Curcumin bound, but gave relatively more negative vina score than 'Curcumin'. However, 'Mahanimbine' and 'Ergosterol' have been found to be slightly violating from the Lipinski's rule of 5 in terms of 'LogP'.

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