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Pharmacotherapeutics and molecular docking studies of alpha-synuclein modulators as promising therapeutics for Parkinson's disease

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Abstract: Parkinson's disease (PD) is an age-related neurodegenerative ailment that affects dopamine-producing neurons in a specific area of the brain called the substantia nigra of the ventral midbrain. It is clinically characterized by movement disorder and marked with unusual synaptic protein alpha-synuclein accumulation in the brain. To date, only a few Food and Drug Administration (FDA) approved drugs are available on the market for the treatment of PD. Nonetheless, these drugs show parasympathomimetic related adverse events and remarkably higher toxicity; hence, it is important to find more efficacious molecules to treat PD. In our study, We chosen 22 natural compounds as inhibitors that potentially block the alpha-synuclein clump—the pathological hallmark of PD—and provide new avenues for its treatment. Most of these molecules exhibited good pharmacokinetic behaviors, making them decisively favorable drug candidates to cure PD. Molecular docking studies were performed to investigate the binding interactions between natural compounds and alpha-synuclein as anti-Parkinson drug targets. Among the examined compounds, curcumin and piperine emerged as promising phytochemicals with the highest binding affinity, key residual stable bindings and showed a good inhibitory features. Thus, the present study indicates that curcumin and piperine hold the potential to be developed as treatment options against PD. Experimental validations are needed for insights into their mechanism of action and potential clinical application.

Introduction

Parkinson's disease (PD) is characterized by a decrease in the number of neurons in the substantia nigra and the formation of Lewy bodies (Zhu and Gong, 2020). PD is the second most common neurodegenerative disease, followed by Alzheimer's disease (M'Angale and Staveley, 2017). PD is accompanied by motor deficit due to selective loss or degeneration of dopaminergic neurons encompasses striatum, substantia nigra, nigrostriatal pathway (Khan *et al.*, 2010). Presently this disorder affects ~1% of the population over 65-year of age (Huang *et al.*, 2012). The accumulation of alpha-synuclein is the pathological hallmark of PD, encoded by the SNCA gene and plaque formation in substantia nigra, also called Lewy bodies (Alecu and Bennett, 2019). Numerous clinical symptoms associated with PD are motor deficits, abnormal movement or bradykinesia, Akinesia, rigidity, resting tremor, and abnormality in gait (Jayaraj *et al.*, 2013). Other symptoms include anxiety, psychosis, sleep disturbances, depression, and memory impairment (Gupta *et al.*, 2018). Oxidative stress plays a key role in the pathogenesis of PD (Wei *et al.*, 2018); it is caused by the overproduction of free radicals, which produces ROS that creates an imbalance between reactive oxygen species and the antioxidant defense system, which can cause damage to DNA, proteins, and lipids (Ghaffari *et al.*, 2018). PD can be categorized into genetically linked and sporadic forms; 90% of the patients suffering from PD carry a sporadic/idiopathic form of the disease, while the remaining are associated with the genetic form. PD linked to genetic form has mutations in genes like LRRK2, Parkin, Pink1, DJ-1, and alpha-synuclein (Lopert and Patel, 2016). Although the root cause is still unknown, several risk factors, such as gender, age,

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and family history, play a crucial role in the development of PD (Roshan *et al.*, 2016).

Moreover, the deficiency of dopamine has been found to be strongly associated with inflammatory response and characterized by reactive microglia with large cell bodies and short process (Cataldi *et al.*, 2016). Various other aspects that increase the occurrence of 'PD are excessive use of herbicides, milk products, and being overweight (Nicholatos *et al.*, 2018). Neuroinflammation is thought to be the main factor in the pathogenesis of PD. The activation of microglia and aggregation of cytokine is found in postmortem PD brains and experimental PD models (Xu *et al.*, 2017; Campolo *et al.*, 2017). The pathogenesis of PD is still not fully explained, but several important intracellular processes are linked, such as mitochondrial dysfunction, endoplasmic reticulum stress, aggregation of misfolded protein, and imbalance between apoptosis and autophagy (Liu *et al.*, 2018). The main factors which cause neuronal death in PD are oxidative stress and mitochondrial dysfunction (Zhang *et al.*, 2010). Genetic evidence shows that a high expression of alpha-synuclein is associated with the pathological process of PD, although its physiological function is poorly known. The soluble oligomer type or fibrillar state of alpha-synuclein can be neurotoxic (Finkelstein *et al.*, 2017).

Alpha-Synuclein is a small protein containing 140 amino acid residues encoded by the SNCA gene with a transcript length of 3041 bp (Siddiqui *et al.*, 2016). The early onset of PD is associated with a missense mutation, and genetic studies have confirmed its involvement in both types of PD (familial and sporadic) (Stefanis, 2012). While the protein is primarily localized in the central nervous system (Oikawa *et al.*, 2016), it is tremendously expressed in the peripheral nervous system and presynaptic terminals of the brain that connect the synaptic vesicle apparatus. The expression of alpha-synuclein is elevated within the synapses of nigral dopamine neurons (Longhena *et al.*, 2017). Plant-derived natural compounds can prevent or inhibit the process of various neurological disorders such as PD and other neurological dysfunction. Naturally occurring phytochemicals such as flavonoids and polyphenols have been shown to possess several pharmacological properties which play an important role in preventing neurodegeneration disorders (Alam *et al.*, 2018).

In this study, various naturally available molecules were selected based on the available literature, and their pharmacological properties, such as antioxidants, anti-inflammatory, antitumor, etc., were examined. The potency of these molecules was compared with another natural molecule based on the molecular docking score. The result revealed these natural molecules as potent inhibitors against PD.

Materials and Methods

A schematic workflow of this study is represented in Fig. 1.

Selection of natural compounds

We selected natural compounds/molecules based on their symptomatic inhibition activity and good pharmacological evidence against various diseases. Selected molecules are potential drug candidates against various oxidative stress-based symptomatic diseases. The chemical structure of all these molecules or compounds was drawn using ChemBioDraw Ultra 12.0 (CambrigdeSoft), as shown in Fig. 2.

Calculation of basic pharmacokinetic parameters

To be considered a novel drug, a compound or molecule must be passed through multiple filters. In this way, most molecules that fail in the preclinical trial are eliminated from the queue of drug molecules (Wolohan and Clark, 2003). Pharmacokinetic

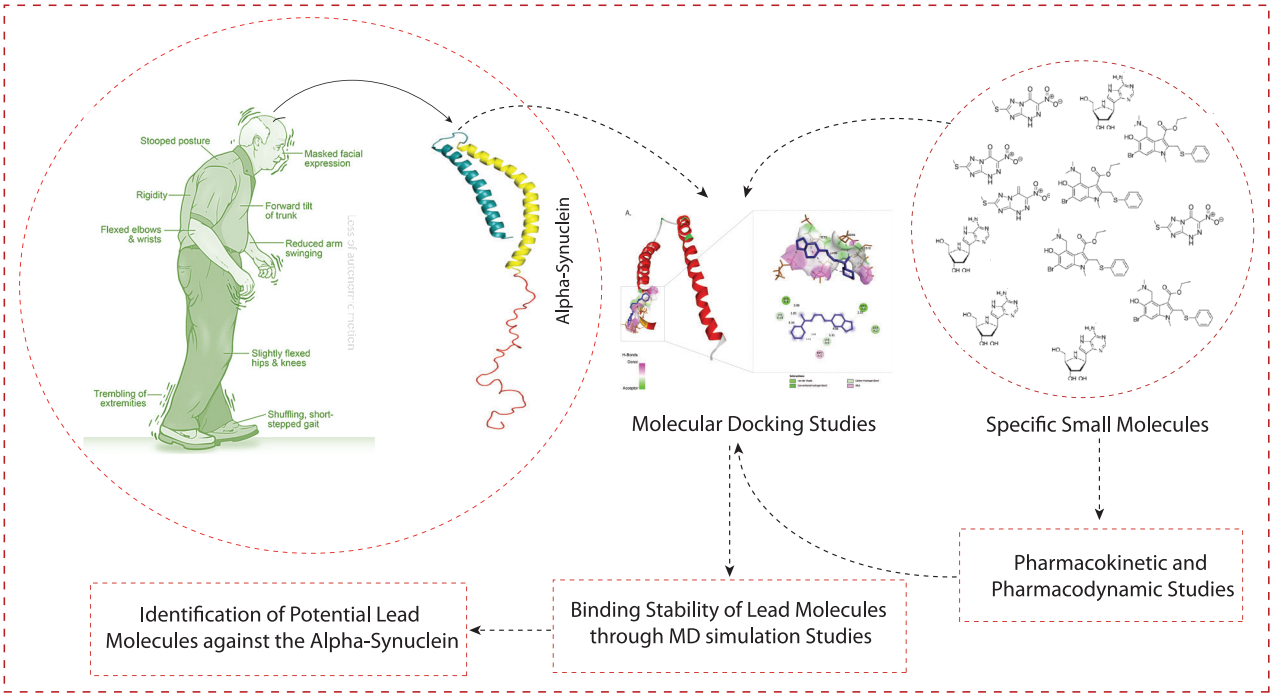

FIGURE 1. The schematic representation of workflow and methodology used in this study.

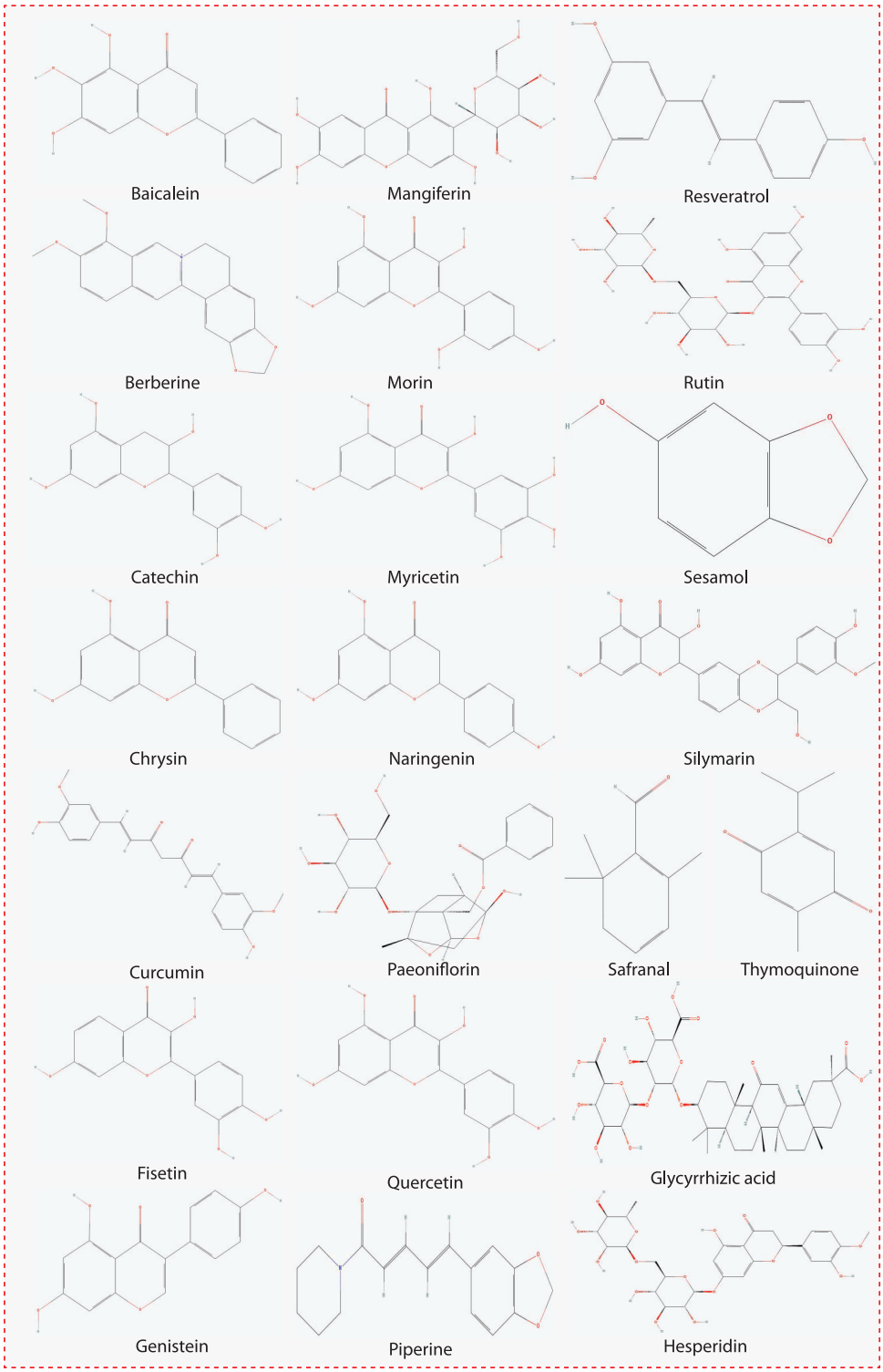

FIGURE 2. The chemical structure of the compounds examined in this study.

properties, including absorption, distribution, metabolism, excretion, and toxicity, play a crucial role in drug discovery and design (Moroy *et al.*, 2012). Physicochemical parameters such as MW, LogP, number of hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA), calculated topological polar surface area (TPSA), number of rotatable bonds (nrtB), and violations of Lipinski's rule of five (n-Violation) was calculated using the *in silico* tool Molinspiration (Stierand and Rarey, 2010).

Prediction of the toxicity compounds

The prediction of toxicity of compounds is integral to drug design and development. *In silico* toxicity predictions are easy and rapid and can also minimize the number of animal usage. So, in this study, LD_{50} was estimated for all the selected natural bioactive markers. The LD_{50} is the lethal median dose required for killing 50% of a test population. It is the measure for evaluating the virulence of the medication or a poison. A lower LD_{50} dose is indicative of increased

toxicity of the substance. Herein, an online tool, ProTox, was used to calculate these LD_{50} values (Drwal *et al*., 2014). Its carcinogenic, mutagenic, skin irritancy properties were determined using Discovery studio 2.5 (Accelrys Software Inc., San Diego, CA, USA) (Anon, 2009).

Target preparation

For this study, the amino acid sequence of the PD-associated target, alpha-synuclein (PDB ID: 1XQ8), was downloaded from the protein data bank (http://www.rcsb.org/pdb/home/home.do). For the crystal structure of targets, the crystallographic water molecules were removed, the missing hydrogen atoms were added, and the energy level of targets was minimized by the Swiss PDB viewer tool (Johansson *et al*., 2012).

Ligand preparation

We selected 22 compounds based on their potential drug-like properties based on earlier reports. The structure of the selected 22 compounds was downloaded from the PubChem database to their 3D form, and ChemBio3DUltra was used to optimize the geometry of the compounds (PerkinElmer Informatics, Waltham, MA, USA). The list of compounds and their sources are listed in Table 1.

Molecular docking and molecular dynamics study

Docking studies generate important information regarding the orientation of the different compounds within the binding pocket of the target protein. In this study, 22 target compounds and four previously reported inhibitors (metaproterenol, clenbuterol, salbutamol, and gallic acid) were used as control (reference) drugs for their inhibition activity against the alpha-synuclein aggregation (Suppl. Fig. S1) were used as control (reference) drugs. All these compounds were docked individually to the alpha-synuclein with a grid center (grid coordination) and grid boxes of certain sizes for each receptor. The flexibility of the compounds was maintained when they interacted with the target protein under rigid conditions. Kollman charges and polar hydrogen atoms were included in the surviving structure. The grid size was set at $126 * 126 * 86$ (x, y, and z) points, and the grid center was designated at x, y, and z dimensions of 249.78, 62.80 and −13.94, respectively, with a grid spacing of 0. 0.333 Å. Ligand-binding affinities were predicted as negative Gibbs free energy (ΔG) scores as (kcal/mol) and calculated using the AutoDock Vina scoring function (Trott and Olson, 2009). The ligand-receptor interaction was visualized using BIOVIA Discovery Studio Visualizer (Anon, 2009), which showed the sizes and locations

TABLE 1

The natural occurrences and chemical formula of compounds selected for this study

S. No.	Compounds name	Mol. formula	Sources
1	**Piperine**	$C_{17}H_{19}NO_3$	Black paper and white paper
2	**Resveratrol**	$C_{14}H_{12}O_3$	Red grape, peanuts, mulberries, blueberries, raspberries
3	**Chrysin**	$C_{15}H_{10}O_4$	Honey, propolis, mushroom, blue passion
4	**Rutin**	$C_{27}H_{30}O_{16}$	Buckwheat, peach, green tea, asparagus, apple, green tea, betula leaf, onion, orange, lemon
5	**curcumin**	$C_{21}H_{20}O_6$	Turmeric
6	**Glycyrrhizic acid**	$C_{42}H_{62}O_{16}$	Licorice
7	**Catechin**	$C_{15}H_{14}O_6$	Green tea, broad beans, apricots, strawberries, pome fruit, cocoa, vinegar, barley, red wine, black grapes
8	**Hesperidin**	$C_{28}H_{34}O_{15}$	Lemon, grapefruits, beverages like tea and red wine, sweet orange (*Citrus sinensis)*
9	**Quercetin**	$C_{15}H_{10}O_7$	Onion, kales, apples, red grapes, berries, capers, broccoli, cherries, and also found in tea and red wine
10	**Morin**	$C_{15}H_{10}O_7$	Fig, white mulberry, almond, sweet chestnut
11	**Fisetin**	$C_{15}H_{10}O_6$	Onion, apple, cucumber, grape, strawberries, persimmon
12	**Naringenin**	$C_{15}H_{12}O_5$	Grapefruits, oranges and kino, tomatoes
13	**Mangiferin**	$C_{19}H_{18}O_{11}$	Mango, kernel
14	**Silymarin**	$C_{25}H_{22}O_{10}$	Milk thistle
15	**Myricetin**	$C_{15}H_{10}O_8$	Tea, berries, vegetable, grapes, wine, walnut, etc.
16	**Thymoquinone**	$C_{10}H_{12}O_2$	Black cumin
17	**Berberine**	$C_{20}H_{18}NO_4$	*Berberis vulgaris*, *B. aristata*, etc.
18	**Genistein**	$C_{15}H_{10}O_5$	Coffee, soybean, fava beans, lupin, kudzu, Psoralea
19	**Sesamol**	$C_7H_6O_3$	Seed coat and oil of roasted *Sesamum indicum*
20	**Baicalein**	$C_{15}H_{10}O_5$	*Scutellaria baicalensis*
21	**Paeoniflorin**	$C_{23}H_{28}O_{11}$	*Paeonia lactiflora*, *Salvinia molesta*
22	**Safranal**	$C_{10}H_{14}O$	Saffron

of binding sites and various types of molecular interactions, including hydrogen-bond, hydrophobic interactions, and bonding distances. Subsequently, the binding poses of each ligand were observed, their interactions were characterized, and the best and most energetically favorable conformations of each ligand were selected and used for molecular dynamics (MD) simulation analysis.

Further, MD simulations were performed using the GROMACS version 5.1 software using the standard GROMOS 53A6 force field (van der Spoel *et al*., 2005). The proteins were soaked in a cubic box of 10 Å dimension containing water molecule, i.e., setting a 10 Å edge for a box from the molecule perimeters, using the *editconf* module for setting boundary conditions and *genbox* for solvation. The solvation of proteins was done using the *spc*216 template. The complete system was neutralized by adding counter ions like Na^+ and Cl^- (for example, to neutralize six positive charges, we added six Cl^- ions, and vice versa for the negative charge, we added six Na^+ ions) using the *gmx genion* module to sustain neutrality. The system was then minimized for 1500 steps at 300 K with an equilibration period of around 100 ps at a constant volume under periodic boundary conditions. Equilibration steps were performed in two phases: NVT ensemble (varying pressure) and NPT ensemble (varying volume). After the equilibration phase, the particle *Mesh Ewald* method was used (Cheatham *et al*., 1995). Production phases of dynamics simulation was performed for 20 ns at a constant temperature of 300 K. The next important thing to analyze was ligand topology. We used SWISSPARAM because it provides topologies and other parameters for small organic molecules compatible with the CHARMM all-atom forced field for use with the CHARMM or GROMACS Software (Bjelkmar *et al*., 2010; Zoete *et al*., 2011). The server is fully automatic and takes the drug molecule in the mol2 format. Topology and parameter result files were retrieved on a registered e-mail ID. For GROMACS users, a .itp file was generated for the small molecule, which was included in the protein topology produced by GROMACS.

Results

Pharmacokinetic properties

The bioactive markers must possess certain properties and pass basic filters like Lipinski's rule of five (RO5) to be considered suitable drug candidates. Evaluation results of drug-likeness properties among selected compounds showed that the highest binding affinity of lead molecules followed Lipinski's RO5, and it has a high potential to be used as a drug. "Lipinski rule of five" is a well-known factor in producing a drug moiety; proven available data from the analysis of the world drug index and helps enhance the drug properties, which possesses all the characteristics to be administered as an orally active drug molecule. This rule is formulated and followed by most orally administered drugs. Four different criteria were used to decide if a molecule is a drug such as (a) having a molecular weight of ≤500 kda, (b) LogP (logarithm of partition coefficient) ≤5, (c) to have ≤5 hydrogen bond donor sites, and (d) hydrogen bond acceptor sites should be ≤10. Molecules that violate the aforesaid rules are believed to have bioavailability-related problems. So given that in our case, most of the compounds passed the RO5 except rutin, glycyrrhizic acid, hesperidin, and mangiferin, which can thus have issues with bioavailability when taken orally.

TPSA measurement was used to determine the bioavailability of all-natural bio-active compounds in the study; it can be performed using Veber's rule. For good oral bioavailability, TPSA measurement says that the molecule must have a rotatable bond, which is ≤10, and the TPSA values ≤140 Å (Veber *et al*., 2002). The number of rotatable bonds signifies the oral bioavailability of drugs. This can distinguish among drug compounds that already have an oral bioavailability. The rotatable bond is explained as any single non-ring bond bound to a non-hydrogen atom. However, sometimes, having more rotational energy barriers also disqualifies rotatable bonds like amide C-N bonds. We performed absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile classification and regression for these 22 molecules; ADME features of compounds are vital for a clear understanding of the pharmacokinetic (PK) properties (Table 2). ADME of a compound is an important parameter; thus, the results of ADME of the 22 examined compounds are presented in Suppl. Table S1.

Toxicity prediction

Toxicity is another important aspect of the drug that needs to be accessed before considering any molecules as a potential drug. We used the online software PROTOX to predict the LD_{50} value of compounds. Expect a few, the bioactive moiety of all compounds in the current study were in non-toxic zone, with LD_{50} of more than 1000 mg/kg mark, except for piperine (330 mg/kg), quercetin (159 mg/kg), fisetin (159 mg/kg), mangiferin (2 mg/kg), myricetin (159 mg/kg), berberine (200 mg/kg), and sesamol (580 mg/kg). Besides, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity were also predicted, and most of the compounds did not show any of the above toxicities, although a few compounds exhibited potentially severe toxicity problems; a detailed analysis of the results is listed in Table 3. The toxicity of the chemical compound was determined in the form of toxicity endpoints such as mutagenicity, carcinogenicity, some other endpoints, etc.

Further, moving up, toxicity may also measure quantitatively (LD_{50}_lethal dose); qualitatively (active or inactive) both as well for special cell types followed by assays. For those compounds assumed as toxicity targets having very little binding possibility, a compound may be active for several toxicity endpoints, resulting in severe toxic effects. Hence, this assay serves as a crucial preclinical trial and is economical and time-saving.

Molecular docking studies

To make sure the binding between the potential bioactive moiety of compounds (Table 3) and PD target (1X08), we performed molecular docking analysis (the score with the inhibition constant is mentioned in Table 4) and two compounds, namely curcumin and piperine show the highest score, bind with a good binding affinity (−9.10 kcal/mol and −8.6 kcal/mol, respectively) and form H-bonds with the residues, e.g., SER 9, MET 1, ASP 2. Piperine with

TABLE 2

The drug-likeness properties of compounds (violation of Lipinski's rule are highlighted in red)

S.No	COMPOUNDS	LogP <5	TPSA	$_n$ATOMS	MW <500	nON <10	nOHNH <5	nROTB <10	nVIOLATIONS
1	Piperine	3.33	38.78	21	285.34	4	0	3	0
2	Resrvetol	2.99	60.68	17	228.25	3	3	2	0
3	Chrysin	2.94	70.67	19	254.24	4	2	1	0
4	Rutin	−1.06	269.43	43	**610.52**	**16**	**10**	6	**3**
5	Curcumin	2.30	93.07	27	368.38	6	2	8	0
6	Glycyrrhizic acid	1.97	267.04	58	**822.94**	**16**	**8**	7	**3**
7	Catechin	1.37	110.37	21	290.27	6	5	1	0
8	Hesperidin	−0.55	234.30	43	**610.57**	**15**	**8**	7	**3**
9	Quercetin	1.68	131.35	22	302.24	7	5	1	0
10	Morin	1.88	131.35	22	302.24	7	5	1	0
11	Fisetin	1.97	111.12	21	286.24	6	4	1	0
12	Naringenin	2.12	86.99	20	272.26	5	3	1	0
13	Mangiferin	−0.16	201.27	30	422.34	**11**	**8**	2	**2**
14	Silymarin	1.47	155.15	35	482.44	10	5	4	0
15	Myricetin	1.39	151.58	23	318.24	8	**6**	1	1
16	Thymoquinone	1.90	34.14	12	164.20	2	0	1	0
17	Berberine	0.20	40.82	25	336.37	5	0	2	0
18	Genistein	2.27	90.89	20	270.24	5	3	1	0
19	Sesamol	1.35	38.70	10	138.12	3	1	0	0
20	Baicalein	2.68	90.89	20	270.24	5	3	1	0
21	Paeoniflorin	0.04	164.38	34	480.47	**11**	5	7	1
22	Safranal	2.95	17.07	11	150.22	1	0	1	0
Reference Drugs									
1	Gallic acid	0.59	97.98	12	170.12	5	4	1	0
2	Metaproterenol	0.55	72.71	15	211.26	4	4	4	0
3	Clenbuterol	2.79	58.28	17	277.19	3	4	4	0
4	Salbutamol	1.35	72.71	17	239.31	4	4	5	0

TABLE 3

Calculated LD50 values and various toxicity predictions of the selected compounds

Compounds	LD_{50} (mg/kg)	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytototoxicity
		Probability	Probability	Probability	Probability
Piperine	330	Active	Active	Inactive	Inactive
		0.53	0.96	0.96	0.53
Resrvetol	1560	Inactive	Inactive	Inactive	Inactive
		0.71	0.86	0.92	0.98
Chrysin	3919	Inactive	Inactive	Inactive	Inactive
		0.62	0.99	0.57	0.87
Rutin	5000	Inactive	Active	Inactive	Inactive
		0.91	0.98	0.88	0.64
Curcumin	2000	Inactive	Active	Inactive	Inactive
		0.84	0.92	0.88	0.88
Glycyrrhizic acid	1750	Inactive	Active	Inactive	Inactive
		0.61	0.99	0.96	0.73

(Continued)

Table 3 (continued)

Compounds	LD_{50} (mg/kg)	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytototoxicity
		Probability	Probability	Probability	Probability
Catechin	10000	Inactive	Inactive	Inactive	Inactive
		0.51	0.96	0.55	0.84
Hesperidin	12000	Inactive	Active	Inactive	Inactive
		0.93	0.99	0.90	0.93
Quercetin	159	Active	In-active	Active	In-active
		0.68	0.87	0.51	0.99
Morin	3919	Inactive	Active	Inactive	Inactive
Fisetin	159	Active	Inactive	Inactive	Inactive
		0.71	0.51	0.53	0.98
Naringenin	2000	Inactive	Inactive	Inactive	Active
		0.62	0.88	0.83	0.59
Mangiferin	2	Inactive	Active	NA	Inactive
		0.72	0.50	…..	0.87
Silymarin	2000	Inactive	Active	Inactive	Inactive
		0.72	0.97	0.69	0.77
Myricetin	159	Active	Inactive	Active	Inactive
		0.68	0.86	0.81	0.91
Thymoquinone	2000	Inactive	Inactive	Inactive	Inactive
		0.63	0.97	0.91	0.78
Berberine	200	Active	Active	Active	Active
		0.56	0.99	0.62	0.96
Genistein	2500	Inactive	Inactive	Inactive	Inactive
		0.69	0.97	0.74	0.91
Sesamol	580	Active	Inactive	Inactive	Inactive
		0.88	0.62	0.73	0.89
Baicalein	3919	active	Inactive	Active	Inactive
		0.68	0.99	0.51	0.99
Paeoniflorin	4000	Inactive	Inactive	Inactive	Inactive
		0.85	0.86	0.81	0.54
Safranal	5000	Inactive	Inactive	Inactive	Inactive
		0.59	0.98	0.80	0.84
Reference drugs					
Gallic acid	2000	Active	In-active	Inactive	Inactive
		0.56	0.99	0.94	0.91
Metaproterenol	205	Inactive	Inactive	Inactive	Inactive
		0.86	0.97	0.85	0.74
Clenbuterol	229	Inactive	Inactive	Inactive	Inactive
		0.67	0.98	0.87	0.61
Salbutamol	660	Inactive	Inactive	Inactive	Inactive
		0.86	0.88	0.75	0.66

Active = Positive; Inactive = Negative

1X08 forms H-bonds with residues LYS 97, PHE 94, and LYS 96 (Fig. 3). Additionally, the molecular docking outcomes near the ligand surrounding amino acid residue (within 4 Å) show polar interaction over a small range that stabilized the complex formation (delocalization of charges with the help of ligands).

TABLE 4

Molecular docking between the selected natural compounds and Parkinson's disease targets (1X08) and the docking scores and inhibition constants

Compound name	Docking score	Inhibition constant
Piperine	**−8.6**	4.96719E-07
Reservetol	−4.4	0.000595325
Chrysin	−5.2	0.000154293
Rutin	−6.8	1.03641E-05
Curcumin	**−9.1**	2.13602E-07
Glycyrrhizic acid	−6.1	3.37782E-05
Catechin	−5.6	7.85493E-05
Hesperidin	−5.6	7.85493E-05
Quercetin	−5.2	0.000154293
Morin	−5.7	6.635E-05
Fisetin	−6.2	2.85322E-05
Naringenin	−5.3	0.00013033
Mangiferin	−5.5	9.29915E-05
Silymarin	−6.2	2.85322E-05
Myricetin	−4.2	0.000834366
Thymoquinone	−4.0	0.001169388
Berberine	−6.0	3.99888E-05
Genistein	−4.9	0.000256006
Sesamol	−4.4	0.000595325
Baicalein	−5.3	0.00013033
Paeoniflorin	−6.3	2.4101E-05
Safranal	−8.5	5.88047E-07
Four reference drugs have been previously reported for their inhibitory activity against alpha-synuclein aggregation.		
Gallic acid	−4.9	0.000256
Metaproterenol	−4.6	0.000424
Clenbuterol	−3.9	0.001384
Salbutamol	−4.1	0.000987

Simulation results of molecular docking

MD simulations of all atoms were performed for 50 ns to understand the mechanism of interaction of curcumin and piperine with 1X08. Several analyses were made using trajectories generated by simulations. The average root means square deviation (RMSD), the root-mean-square fluctuation (RMSF), solvent accessible surface area (SASA), and radius of gyration (Rg) were estimated, and the results are mentioned in Table 5.

Structural deviations and compactness

To evaluate conformational changes of alpha-synuclein upon ligand binding, RMSD was determined to estimate the stability of the system under-exploited solvent conditions. The RMSD plot suggested that the binding of curcumin and piperine stabilized 1X08 and led to fewer structural deviations from its native conformation. In Fig. 4A, curcumin and 1X08 complexes showed distinct deviation until 25 ns from the starting point. However, the complex appeared to stabilize until the end of the simulation. Although piperine followed the same pattern of deviation in this study, both the curcumin and piperine were bound perfectly into the cavity of 1X08 protein and stabilized the system; this stability is necessary to stop the action of proteins after the binding of curcumin and piperine.

Root-mean-square fluctuation

To calculate the average fluctuation of all residues during the simulation, the RMSF of the binding of IX08 upon ligands was plotted as a function of residue number, as shown in Fig. 4B. RMSF plot showed the presence of several residual fluctuations in different regions of the IX08 structure. These residual fluctuations were found to increase upon binding of curcumin and piperine in the region spanning in N-terminal to C-terminal and affect the dynamics behavior of residues. RMSF values fluctuated highly in IX08-curcumin, starting from residue 48, and in IX08-piperine, after residues 65. These findings indicate that the IX08 complexes have smaller RMSF values than the native IX08 structure. Thus, the observed drifting was accompanied by increased atomic fluctuations in the complex structure. The major fluctuations suggested the profound importance of these residues in the ligand-binding process.

The radius of gyration (R_g)

R_g is linked to the three-dimensional volume of a protein and is applied to assess the stability of the protein in a biological system. A protein is supposed to have a higher R_g due to less tight packing. Here in our study, the Rg plot suggested more tight packing of 1X08 upon the binding of curcumin and piperine Fig. 4C. Minimal structural deviation can be seen in the Rg plot, and no conformational shift was observed after the binding of both drugs. The Rg plot showed a tight packing of 1X08 proteins from the initial to the endpoint of simulation after the binding of drugs, suggesting that both the drugs were bound tightly with the proteins and may stop the action of proteins.

Solvent accessible surface area

SASA is a protein's surface area interacting with its solvent and indicates the folding-unfolding state and stability of the protein. The average SASA values for IX08-curcumin and IX08-Piperine were monitored during 50 ns MD simulations. The complexes presented here showed a lowered value of SASA with time, while the native IX08 structure showed a slightly larger SASA value Fig. 4D.

Discussion

PD is the second-fastest-growing neurodegenerative disorder worldwide after Alzheimer's disease and highly affects the elderly (Cornejo *et al.*, 2021). The alpha-synuclein oligomer and fibrils are crucial for PD pathogenesis. A previous study has shown that the toxicity is caused by oligomers and dopaminergic cell death in the rat model of PD (Cascella *et al.*, 2021). Hence an effective therapeutic approach could be by inhibiting the oligomerization of toxic oligomers into fibrils. Many recent studies have suggested the reduction of toxic oligomers via the modulation of natural compounds. In this perspective, curcumin has been shown to be effective against

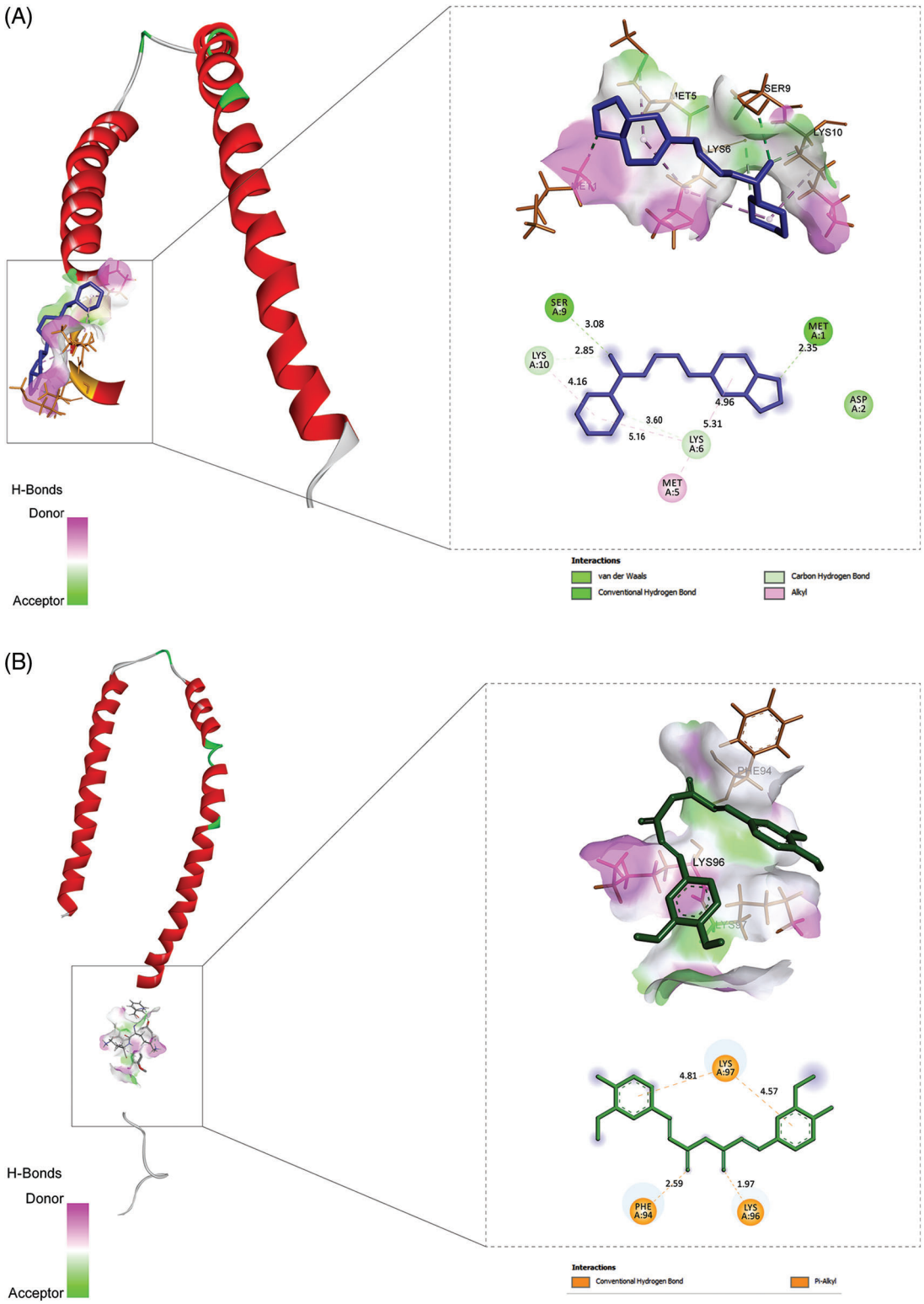

FIGURE 3. The results of molecular docking studies: (A) Piperine and 1XQ8 (B) Curcumin and 1XQ8.

various diseases such as cancer, diabetes, cardiovascular, and other neurological disorders and might be a promising therapeutic agent. Curcumin possesses antioxidant and anti-inflammatory properties and detoxifies the effects of reactive oxygen species (Jha *et al.*, 2016). It is a natural compound mainly found in the root of the *Curcuma longa* plant and is the main component of turmeric spices. Curcumin is biologically most active and abundant among the three curcuminoids (Spinelli *et al.*, 2015). Research has proven that this compound possesses antioxidant, anti-inflammatory, cardioprotective, neuroprotective (Wang *et al.*, 2020), anti-apoptotic (El Nebrisi *et al.*, 2020), and chemopreventive (Scapagnini *et al.*, 2011) properties. It also exhibits free radical scavenging activity, and such properties could combat nitrative and oxidative stress in various neurodegenerative disorders like PD and Alzheimer's disease. Studies have strongly favored the clinical manifestation of curcumin in a different model of PD (Hu *et al.*, 2015).

TABLE 5

Average values calculated for the systems obtained after 50 ns molecular docking simulations (RMSD, root means square deviation; RMSF, root-mean-square fluctuation; SASA, solvent accessible surface area

Protein/complex	Average RMSD (nm)	Average RMSF (nm)	Average Rg (nm)	Average SASA (nm^2)
1XQ8	3.18203	1.27388	2.28472	99.03
1XQ8-Piperine	3.40318	1.58914	2.18194	95.5268
1XQ8-Curcumin	3.05294	1.42524	2.41245	96.7144

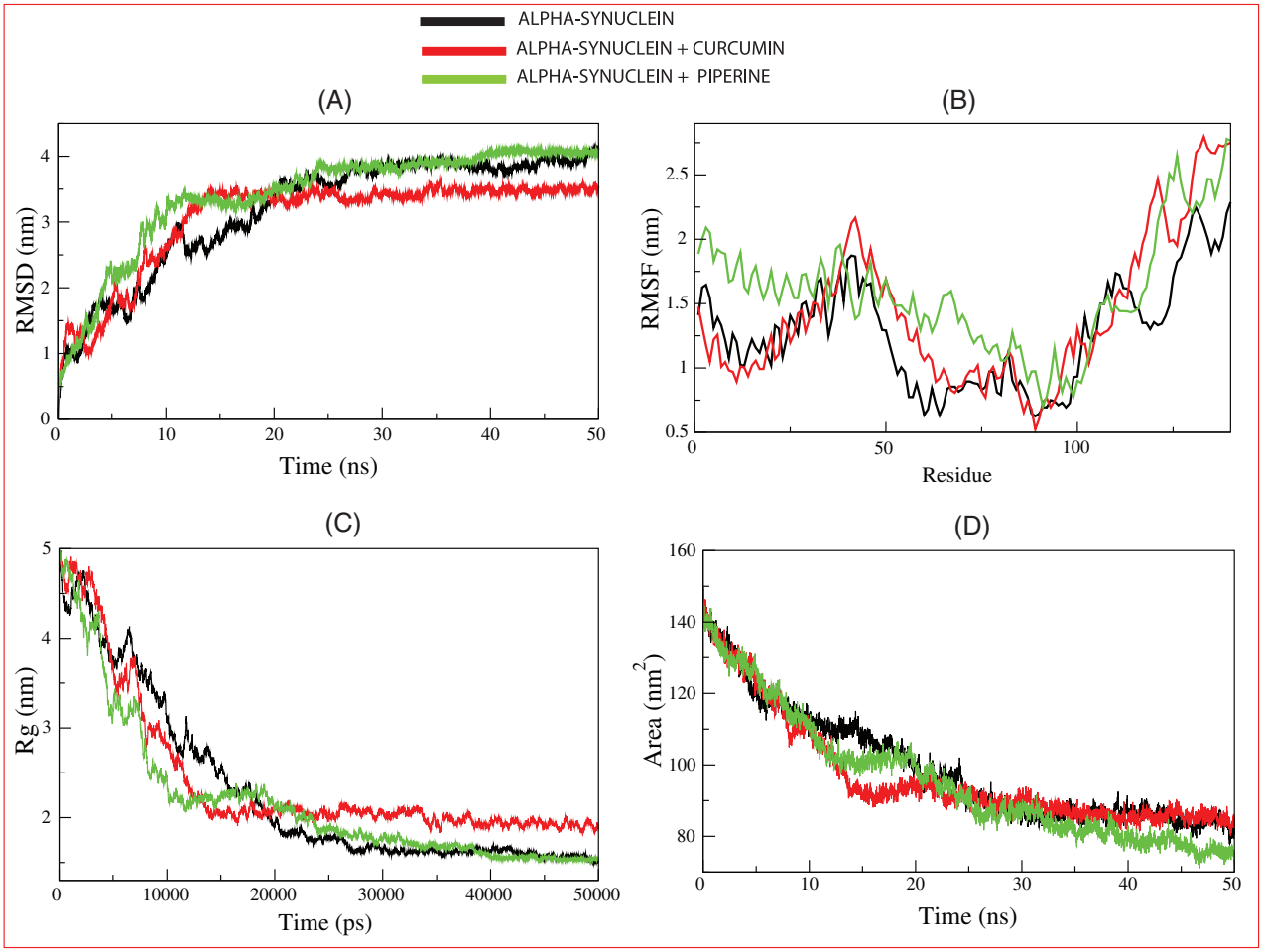

FIGURE 4. The molecular dynamic simulation of the lead compounds with the Parkinson disease target: (A) root means square deviation (RMSD), (B) root-mean-square fluctuation (RMSF), (C) Radius of gyration (Rg), and (D) solvent accessible surface area (SASA).

Besides, in one study, piperine attenuated the memory performance and enhanced myelin repair in Lys phosphatidylcholine-induced animal model of a rat via anti-inflammatory, antioxidants, and neuroprotective effects. Therefore, treatment with piperine increased the level of antioxidant capacity. Evidence-based experimental findings have proven that piperine is a promising therapeutic target for myelin repair in multiple sclerosis and improves memory performance (Roshanbakhsh *et al.*, 2020). It is also effective against intracerebroventricular cognitive impairment induced by streptozotocin injections. Piperine could improve memory impairment and decrease amyloid-beta deposition through a neuroprotective antioxidant mechanism (Wang *et al.*, 2019) and exhibit various pharmacological properties such as antidepressant, cognitive analgesic, anti-inflammatory, cytoprotective, and antioxidant effects. Compared with chemical entities, piperine has many beneficial properties, such as easily available natural plant material, low cost, well known, and safe to use (Ashour *et al.*, 2016).

The impairment of the antioxidant defense mechanism is caused by enhanced oxidative stress in the brain and peripheral tissues in Alzheimer's disease, amyotrophic lateral sclerosis, and PD. To date, available treatments for neurodegenerative diseases are not appropriately effective and have side effects. The standard gold treatment of PD using levodopa has copious side effects (Cornejo *et al.*, 2021). Most of the drugs have failed to inhibit or limit the progress of Perkinson's disease (Niedzielska *et al.*, 2016). There is a global need for a new therapeutic alternative

treatment for neurodegenerative disease and PD. Curcumin has been reported to possibly cross the blood-brain barrier and lead to several improvements in the pathological process of neurodegenerative disease (Salehi *et al*., 2020).

Molecular docking, homology modeling, and MD simulation are proven tools for the identification of potent inhibitors. Its approaches are frequently used to understand ligands-receptor interaction in computational drug designing. Apart from the *in vitro* and *in vivo* studies, these computational techniques strongly support manufacturing or designing novel drugs in the pharmaceutical area by deciphering the mechanism of drug-receptor interaction (Srivastava *et al*., 2010; Khan *et al*., 2021). In this study, curcumin and piperine were found to be comparatively more suitable ligands. The result suggested that the stability of the proteins increased after docking, and both the ligands were present for a longer period in the pocket of the protein (Table 4). Herein, curcumin and piperine were observed to bind to the active site of the alpha-synuclein protein and inhibit the self-association of targeted protein to act as drug candidates for PD. Earlier studies suggest diverse pharmacological properties of curcumin and piperine (Yu *et al*., 2017). Alpha-synuclein is the most characteristic protein of PD; therefore, it has important applications in therapeutics and clinical diagnosis (Dehay *et al*., 2015).

Conclusion

The present *in silico* study gives a better insight into the structure and function of alpha-synuclein. Several plant-based compounds are combined with cognitive protective compounds or molecules to improve neurological disorders in PD patients. Our current study expands the understanding of natural molecules or compounds, showing their potential in treating PD. Most importantly, we found two compounds (curcumin and piperine) as variable multi-target-directed compounds with antioxidant and neuroprotective activities. So, these molecules may be prospective therapy for PD and other neurobehavioral diseases. Further studies are crucially important to explore the specific targets in various brain regions and the mode of action, including the signaling pathway, blood-brain barrier (BBB), and mechanism of synergetic effect of the antioxidant agent on the target. This pharmaco-chemical approach can open new avenues to design effective, potential herbal moieties for the treatment of PD with negligible side effects.

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Availability of Data and Materials: All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Supplementary Material: The supplementary table is available online at DOI: 10.32604/biocell.2022.021224.

Authors' Contributions: RA and SKR conceived the study design and analyzed the data. R Ali curated data and performed statistical analyses. AA curated data and prepared figures. R Ali and AA performed molecular docking studies. All the authors read, edited, and approved the final manuscript.

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Supplementary Materials

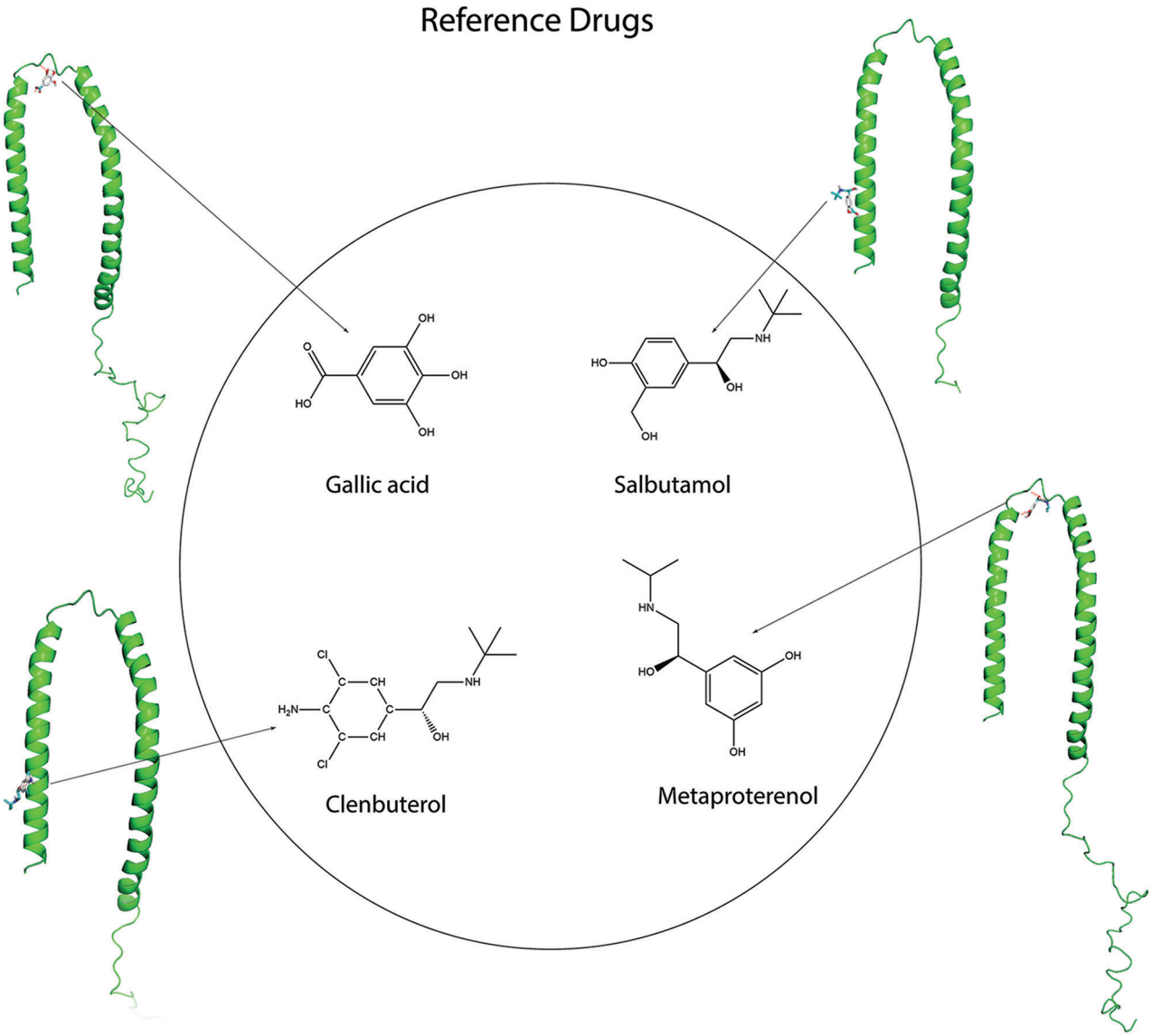

FIGURE S1. Four reference drugs (as control) used for their inhibition activity against the alpha-synuclein aggregation. So, these four compounds were docked individually to the alpha-synuclein and compared the binding affinity with our target compounds.

Table S1. The results of ADME of the 22 examined compounds