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In Silico Screening of GC-MS Identified Phytochemicals from *Ziziphus oenoplia* (L.) Mill Leaves Extract Against Key Parkinson's Disease Targets

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Abstract

Background: Parkinson's disease (PD) is the second most common neurodegenerative disease, which causes α-synuclein aggregation, neuroinflammation, mitochondrial dysfunction, and kinase dysregulation leading to motor and non-motor symptoms. Plant-based compounds hold a superior role for multi-target action on PD pathogenesis, offering safety, alleviating side effects like dyskinesia and hallucinations caused by current PD drugs, and also providing synergistic potential over conventional single-target therapies. **Aim:** The purpose of this study was to investigate the anti-Parkinsonian activity of *Ziziphus oenoplia* (L.) Mill against key pathological targets in the Nrf2 (Nuclear factor erythroid 2-related factor 2) pathway by computational docking. **Methods:** From GC-MS(Gas chromatography-mass spectrometry) data, the thirteen phytochemical compounds from *Ziziphus oenoplia* (ZO) were docked against PD proteins such as Glycogen synthase kinase-3β (GSK-3β), Kelch-like ECH-associated protein 1 (KEAP1), Leucine-rich repeat kinase 2 (LRRK2), Nuclear factor kappa B (NFκB), and α-synuclein against Levodopa as a standard compound using AutoDock Vina. Furthermore, ADMET analysis was done to assess the drug likeness, pharmacokinetic, and toxicity profile of the phytoconstituents using SwissADME and ProTox-3.0 tools. **Results:** Hydroxydehydrostevic acid emerged as the top performer against Levodopa in PD targets. The most notable binding energies are -10.58 kcal/mol for KEAP1, -9.71 kcal/mol for NFκB, and -9.61 kcal/mol across the GSK-3β target. **Conclusion:** This is a preliminary computational study, and additional *in vitro* (*e.g.*, SH-SY5Y neuronal cells for neuroprotection, cytotoxicity, and antioxidant assays) and *in vivo* (MPTP- or 6-OHDA-induced animal models) studies are needed before clinical translation to demonstrate the efficacy of hydroxydehydrostevic acid and to support its progression as a lead compound beyond Levodopa symptom control.

Major Findings: Hydroxydehydrostevic acid outstands Levodopa with superior binding energies and multi-target potential, which highlights the ZO neuroprotective potential.

Keywords: Hydroxydehydrostevic Acid, Levodopa, Molecular Docking, Parkinson's Disease

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1. Introduction

Parkinson's Disease (PD) is the second most prevalent neurodegenerative disease, and it affects up to ten million individuals globally[1]. PD may be described by degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the accumulation of misfolded α-synuclein (α-syn) protein[2]. While unfolded, non-globular α-synuclein is widespread in the human brain, its stable tetrameric forms remain resistant to aggregation in aqueous solutions. When interacting with negatively charged lipids, *e.g.*,

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phospholipids, which constitute cell membranes, α-synuclein folds into α-helical structures (specifically at the N-terminal 111 region)[3]. In PD, α-synuclein undergoes a conformational change to a β-sheet-rich amyloid-like form that easily aggregates. Indeed, misfolded α-synuclein is found within Lewy bodies as 5–10 nm long filaments.

Various mechanisms have been suggested that underlie the conformational changes resulting in abnormal aggregation of α-synuclein, such as phosphorylation at serine 129, ubiquitination and C-terminal truncation. Thus, various forms of α-synuclein are present in the PD brain, such as unfolded monomers, soluble oligomers, protofibrils and high molecular weight insoluble fibrils[4]. Recent work showed that the early oligomeric form of α-synuclein, rather than the mature insoluble fibrils, is the most neurotoxic. The increased toxicity of these oligomers, as opposed to the fibrillary α-synuclein, was validated in cell-based assays. The oligomeric species of α-synuclein are capable of "seeding" and accelerating abnormal protein aggregation, and this might be the mechanism underlying the spread of α-synuclein pathology in the brain[5].

Mitochondrial dysfunction plays a pivotal role in the pathogenic process of both sporadic and inherited PD. Complex-I deficiency in the electron transport chain has consistently been found in the substantia nigra pars compacta in postmortem pathological examination, and in peripheral tissue, such as platelets and skeletal muscle[6]. The mitochondrial impairment model is based on the specificity of dopamine neurotoxins to damage dopaminergic neurons; its metabolically activated accumulation of MPP^+ via the dopamine transporter, and a decrease in complex-I activity. Pharmacological inhibitors of complex-I (*e.g.*, rotenone and paraquat) can also cause this Parkinsonian phenotype[7]. The gene structure of familial PD also suggests the involvement of mitochondrial homeostasis, as both PINK1 and parkin (PARK2/PARK6) encode important components of the mitophagic quality control pathway; mutations causing their loss of function are associated with an early onset, autosomal recessive form of PD, which shows dopaminergic vulnerability[8].

PD pathogenesis is heavily driven by proteostatic network failure, which is interconnected with a pathogenic axis that embraces two dysfunctions: the ubiquitinated-proteasome system and the autophagy-lysosome pathway[9]. The ubiquitin-proteasome system degrades short-lived misfolded proteins via ubiquitin tagging and 26S proteasomal hydrolysis and is abnormally defective in the Parkinsonian substantia nigra, along with reduced expression of the 20S α-subunit and proteasomal activators PA700 and PA28. Genetically validated mutations in genes involved in the ubiquitinylated proteasome system (PARK2, parkin E3 ubiquitin ligase and PARK5, ubiquitin C-terminal hydrolase) support these insufficiencies of the proteasome[10]. At the same time, flux through the autophagy-lysosome pathway is impaired, as evidenced by increased autophagosome levels, as measured by microtubule-associated protein 1 light chain 3, form II, decreased levels of the membrane protein in the lysosome-associated membrane type protein type 2A (LAMP2A), and decreased levels of the two chaperones (Heat Shock Protein 70 and 35) which mediate protein entry into the lysosomes[11]. Mutations of the GBA1 gene, the most potent genetic risk factor for PD, do so by similar mechanisms, that of lysosomal dysfunction, while loss-of-function mutations of the ATP13A2 (PARK9) gene precipitate lysosomal dysfunction, which causes Kufor-Rakeb syndrome[12].

Microglial activation, upregulation of the complement system, infiltration of T lymphocytes, and increased concentrations of pro-inflammatory cytokines have been observed in the substantia nigra and striatum during the neuropathological examination[13]. Microglial activation occurs in the brainstem and basal ganglia at the onset of the disease, and in the cortex in PD patients, as demonstrated using Positron emission tomography (PET) imaging utilising the [^{11}C]-PK11195 radioligand. Importantly, α-synuclein can directly stimulate microglia in a dose-dependent manner, and microglial inhibition with minocycline blocks dopaminergic neurodegeneration in toxin rodent models[14]. Results of the genome-wide association study support immunogenetic susceptibility (Human Leukocyte Antigen class II region), and Ibuprofen utilisation is related to a decreased risk of Parkinsonism (epidemiology). Incorporation of these pathogenic mechanisms: oligomerisation of α-synuclein, failure of bioenergetic function in mitochondria, failure of proteostatic function, and amplification of neuroinflammation, leads to selective

vulnerability of nigrostriatal dopaminergic neurons, and clinical motor evidence only becomes apparent when about 60% of striatal dopamine is depleted[15].

PD is clinically evidenced by cardinal motor symptoms that include tremor, bradykinesia (slow movement), muscle rigidity, instability, and lack of coordination in muscle movement. The non-motor symptoms are psychosis, depression, anxiety, fatigue, and dementia, especially among patients aged 40 years and above[16]. The major pharmacological management is to slow down or prevent the neurodegenerative process and to improve the motor symptoms. The current management includes Levodopa/Carbidopa, Monoaminoxidase-B (MAO-B) inhibitors, catechol-O-methyltransferase (COMT) inhibitors, Dopamine agonist (Both Ergoline and Non-ergoline classes), N-methyl-D-aspartate (NMDA) receptor antagonist, and anticholinergic drugs[17,18]. There are several disadvantages in the current drug management, such as long-term use of Levodopa, which causes motor fluctuations and dyskinesia. Monoamine oxidase-B (MAO-B) Inhibitors (Selegiline, Rasagiline, and Safinamide) inhibit flavin-dependent oxidative deamination of synaptic/extra-synaptic dopamine, increasing intrasynaptic tone and enhancing neuroprotection[19]. The Catechol-O-methyltransferase (COMT) inhibitors (Entacapone, Opicapone, Tolcapone) are Levodopa potentiators that prevent peripheral oxidation of Levodopa to 3-O-methyldopa, and therefore prolong striatal dopamine release and minimise off-time symptoms like dyskinesia and other motor complications. Meanwhile, certain cautions should be noted while administering Entacapone (must be taken as a single dose) and Tolcapone (mandates rigorous hepatotoxicity surveillance due to the risk of acute liver failure)[20,21].

Dopamine agonists that act on D2 receptors are associated with serious adverse effects, such as the ergoline class of drugs, which causes retroperitoneal, pulmonary, and pericardial fibrotic reactions. To overcome this, in the present, non-ergoline drugs are used for managing PD. Apomorphine, which targets the D1 and D2 receptors, reduces motor impairment and Levodopa-induced dyskinesia. The major adverse effect of Apomorphine is drug-induced hallucinations, which are seen in elderly patients with cognitive impairment[22]. Anticholinergic drugs like Benztropine, Diphenhydramine, Orphenadrine, Procyclidine, and Trihexyphenidyl maintain the balance between dopaminergic and cholinergic neurological pathways, and this drug leads to side effects like dry mouth, blurred vision, urinary retention, and constipation[23]. Amantadine and Memantine are NMDA receptor antagonists to manage the motor symptoms of PD, leading to potential adverse effects like nausea, dizziness, insomnia, hallucinations, confusion, and livedo reticularis[24]. To overcome overall drug-caused side effects in PD patients, introducing plant-based drug discovery is a growing trend to address the healthcare challenges.

Empirical studies have justified that a range of ethnopharmacological botanicals and their phytoconstituent metabolites possess potent redox-modulatory as well as anti-inflammatory actions that place them into paradigmatic neuroprotectants of dopaminergic nigrostriatal pathways in the pathogenesis of PD (*e.g.*, curcumin of *Curcuma longa*, resveratrol of *Vitis vinifera*, ginkgetin of *Ginkgo biloba,* etc.)[25,26,27]. *Ziziphus oenoplia* (L.) Mill (ZO), commonly known as Jackal jujube, belongs to the *Rhamnaceae* family, and is found throughout tropical and subtropical parts of Asia, Africa, and Australia. It is a spiny perennial shrub (1–3 m tall) with 3-basal veins on the leaf, which are characteristic; the leaf margin is serrate, the adaxial surface of the leaf is glossy, and all leaves alternate, all of these making leaf collection and standardisation easier for phytochemical extraction[28]. GC-MS and phytochemical studies of ZO leaves and aerial parts have revealed diversified bioactive constituents such as cyclopeptide alkaloids (ziziphin type alkaloids); flavonol glycosides (quercetin, rutin); triterpenoid saponins and polyphenols, tannins, sterols (γ-sitosterol) and various fatty acid derivatives[29]. Together, these compounds have multi-valent bio-functions that are associated with neuroprotection. The traditional health uses of ZO are for treating fever, gastrointestinal disturbances, pain, inflammation, skin infections, and wounds. Ethnomedicinal reports are of anti-inflammatory, analgesic, antidiabetic, hepatoprotective, anthelmintic, antibacterial, and wound healing properties. Pharmacological studies highlight several antioxidant activities (DPPH, FRAP, and lipid-peroxidation inhibition), anti-inflammatory properties (oedema reduction

and decrease in the production of proinflammatory mediators),cytoprotective/hepatoprotective properties, antidiabetic and lipid-lowering properties, and antimicrobial/analgesic activities[30]. These multi-modal activities indicate that there is modulation of oxidative stress, mitochondrial dysfunction, protein aggregation, and neuroinflammation – core mechanisms in PD pathogenesis. In particular, the cyclopeptide alkaloids and the flavonoid conjugates are notable multi-target ligands against PD-related proteins and have blood-brain barrier transport across.

The present study aims to perform *in silico* screening of GC-MS-identified phytochemicals of the ZO leaf extract against targets in Parkinson's disease. Specifically, the GC-MS profile was performed on ZO leaves to identify bioactive phytoconstituents, the molecular docking was subsequently performed against the PD relevant protein targets such as α-synuclein, GSK-3β, and other key enzymes/proteins involved in dopaminergic neurodegeneration; the binding energies, RMSD and the pattern of ligand–receptor interactions were evaluated to prioritise the molecules as potential neuroprotective agents; the ADMET properties were assessed to predict possible drug-likeness and brain penetration potential. This integrated computational strategy seeks to offer a mechanistic and target-driven rationale for moving on ZO phytochemicals as leads in PD drug discovery and to guide future nanoformulation and preclinical validation strategies.

2. Materials and Methods

2.1 Collection and Preparation of Plant Extract

ZO specimens were collected on the 20th of November 2025 from the Auroville area in Puducherry. The identification of ZO (Family: *Rhamnaceae*) was done through the herbarium at the Kanchi Mamunivar Government Institute of Postgraduate Studies and Research (Autonomous), Lawspet, Puducherry – 605008, under the guidance and supervision of Dr. V. Kumaresan, M.Sc., PhD, Assistant Professor of Botany. After collection, 100 g of dried leaves of *Z. oenopolia* were subjected to shade desiccation at 28±2°C, grinding to 60-mesh size, and continuous Soxhlet ethanolic extraction (1:10 w/v ratio with the use of ethanol as a solvent; 6-8 hours reflux), to obtain a concentrated crude product[31].

2.2 Preliminary Phytochemical Screening

Phytochemical characterisation of ZO extracts employed chromogenic diagnostics targeting specific metabolomic classes: Dragendorff's assay (Alkaloids), Molisch's test (Carbohydrates), Keller-Killiani test (Cardiac glycosides), Ninhydrin test (Amino acids), ammonia test (Flavonoids), ferric chloride (Phenolics), Braymer's emulsion test (Tannins), Salkowski's test (Steroids), copper acetate test (Diterpenes), alcoholic KOH (Quinones), Borntrager's test (Anthraquinones), and Hydrochloride test (Anthocyanins)[32]. Subsequently, gas chromatography-mass spectrometry (GC-MS) analysis was conducted at the VIT Vellore Sophisticated Instrumentation Facility and confirmed key metabolites for pharmacological evaluation.

2.3 Gas Chromatography and Mass Spectrometry (GC-MS) Analysis

The study was conducted to determine the bioactive phytoconstituents present in the ZO leaves extract by using GC-MS. It was performed at the VIT, Vellore-632-014, Tamil Nadu, at the highly sophisticated instrumentation facility (SIF), School of Advanced Sciences. The National Institute of Standards and Technology (NIST) mass spectral database includes over 62,000 patterns that were used to match the spectrum of the unknown substances with the known fragment data. The GC-MS spectrum was analysed with the Clarus 680 GC instrument, with Helium as carrier gas, using a fused silica column packed with Elite-5MS (5% biphenyl, 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 µ). The temperature of the injector was kept at 260°C during the chromatographic run. The extracted sample of 1µL was injected into the instrument; the oven temperature was as follows: 60 °C (maintained for 2 min), followed by 300 °C at the rate of 10 °C/min, and 300 °C, where it was held for 6 min. The mass detector conditions were set to 240 °C for transfer line temperature; ion source temperature at 240 °C; and 70 eV for ionisation mode electron impact. The fragments with masses ranging from 40 to 600 Da were set during a scan time of 0.2 s and with an interval of 0.1 s[33].

2.4 Molecular Docking

2.4.1 Selection of Ligands and Protein Targets

The chemical structures of the thirteen identified phytoconstituents of ZO leaves were obtained from the National Centre for Biotechnology Information (NCBI) PubChem database[34] in two-dimensional format (2D). They were then converted into three-dimensional (3D) coordinates and energy minimised by the software suite Open Babel. The 3D protein targets are selected for molecular docking studies, based on their roles in the Nrf2-mediated oxidative stress response pathway, namely KEAP-1 (PDB ID: 4IFJ), leucine-rich repeat kinase 2 (LRRK2) (PDB ID: 6XAF), α-synuclein (PDB ID: 8A9L); pro-inflammatory modulators of Nrf2, glycogen synthase kinase-3β (GSK-3β) (PDB ID: 1J1B) a negative Nrf2 regulator, and nuclear factor kappa B (NF-κB) (PDB ID: 5E69) which crosstalk with Nrf2 signalling. Crystal structures of target proteins were downloaded from the RCSB Protein Data Bank (PDB)[35]. After performing the docking simulations using AutoDock Vina, the binding free energies (ΔG) and inhibition constants (Ki) were calculated; the key residue interactions between hydrogen bonds, hydrophobic contacts, π-π interactions, π-cation interactions, and van der Waals forces were analysed in AutoDock. The specific spatial configurations, binding energies, and individual receptor-ligand interactions were characterised with BIOVIA Discovery Studio Visualizer version 2025[36].

2.5 Pharmacokinetics and Toxicity Analysis

The Swiss Institute of Bioinformatics profiled using the SwissADME web server[37], which is a free online software to assess the physicochemical, lipophilicity, water solubility, pharmacokinetics, drug likeness, and medicinal chemistry. For evaluating the safety margins of the phytoconstituents, computational profiling was executed utilising the machine-learning algorithmic models of the ProTox-3.0's 61 endpoints, GHS acute toxicity classification, and Tox21 stress/Molecular Initiating Events (MIE) pathways[38].

3. Results and Discussion

3.1 Preliminary Phytochemical Screening

The ethanolic leaf extract of ZO was subjected to preliminary phytochemical analysis, which indicated the presence of alkaloids, cardiac glycosides, amino acids, flavonoids, phenolics, steroids, diterpenes, quinones, anthraquinones, and anthocyanins. The presence of tannins and carbohydrates was not detected under the experimental conditions. Alkaloids, flavonoids and phenolics are of special importance in neurodegenerative disorders[39]. The activity of the dopaminergic and cholinergic pathways is shown to be crucially involved in Parkinson's disease (PD) and is reportedly modulated by alkaloids obtained from *Ziziphus* species. The flavonoids and phenolics have strong anti-inflammatory and antioxidant properties, such as free radical scavenging, microglial anti-activating properties, and α-synuclein aggregation inhibition[40]. These properties are similar to the pathophysiological mechanisms involved in PD, which include oxidative stress and neuroinflammation. The results revealed the presence of cardiac glycosides, steroids, diterpenes, and quinones, which suggests the extract could be chemically diverse and have multi-target activity. Some of the classes, however, like cardiac glycosides, might be unsafe, and assessment of ADMET must be performed, and toxicological studies must be done carefully.

3.2 The GC-MS Analysis of ZO Leaf Extract

The chromatogram showed the presence of L-[−]-4-hydroxy-1-methylproline as the major constituent (23.53% area %), 1,6;2,3-dianhydro-4-deoxy-β-D-lyxo-hexopyranose (15.95 %), 1,6;2,3-dianhydro-4-deoxy-β-D-ribo-hexopyranose (13.16 %), phytol (12.43 %) and hydroxydehydrostevic acid (11.34 %), illustrated in Figure 1. Meanwhile, the phytoconstituent identification was performed using the NIST (2008) library; 13 non-redundant phytoconstituents were chosen for further *in silico* evaluation, as shown in Table 1.

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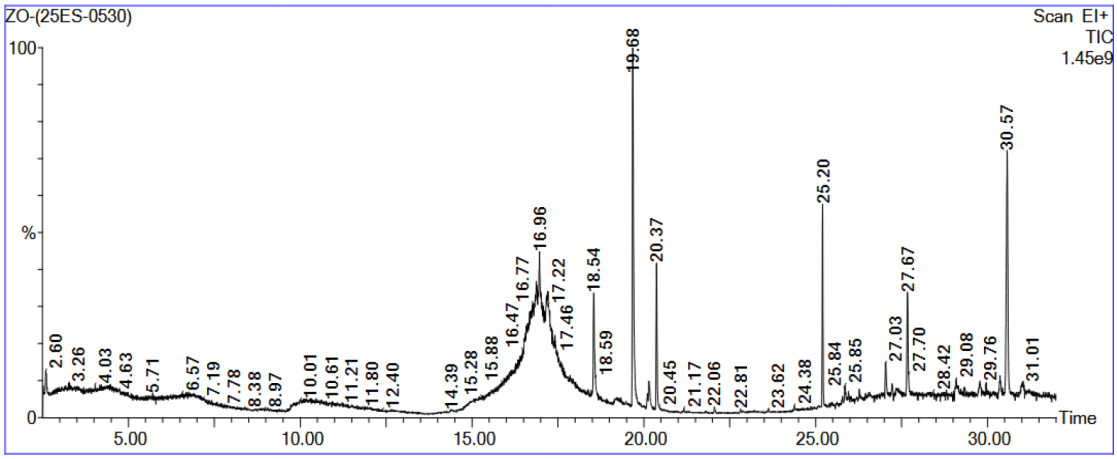


Figure 1. GC–MS chromatogram showing peak area percentages of phytoconstituents in *Ziziphus oenopolia* leaf extract.

Table 1. Phytoconstituents identified from ZO leaves extract via GC-MS analysis

Sr. No.	Identified Phytoconstituents	Molecular Formula	Molecular Weight (g/mol)	Retention Time (RT)	Scan Height	Peak Area %
1	Ethanediamide	$C_2H_4N_2O_2$	88.07	2.603	21	0.867
2	DL-3-Aminobutyric acid	$C_4H_9NO_2$	103.12	4.474	395	1.262
3	L-Guanidinosuccinimide	$C_5H_7N_3O_2$	141.13	6.745	849	1.024
4	L-[-]-4-Hydroxy-1-methylproline	$C_6H_{11}NO_3$	145.16	16.869	2873	23.534
5	1,6;2,3-Dianhydro-4-deoxy-β-D-ribo-hexopyranose	$C_6H_8O_3$	128.13	16.964	2892	13.159
6	1,6;2,3-Dianhydro-4-deoxy-β-D-lyxo-hexopyranose	$C_6H_8O_3$	128.13	17.199	2939	15.948
7	Heptanoic acid, 2,4-dimethyl-, methyl ester, (2S,4R)-(+)-	$C_{10}H_{20}O_2$	172.26	18.545	3208	3.744
8	Phytol	$C_{20}H_{40}O$	296.54	19.675	3434	12.427
9	Pyruvic acid, 3-hexenyl ester	$C_9H_{14}O_3$	170.21	20.146	3528	0.753
10	3,7,11-Tridecatrienenitrile, 4,8,12-trimethyl-	$C_{16}H_{25}N$	231.38	25.203	4539	4.608
	2,4-Dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c)triazine	$C_6H_7N_5O$	165.15	27.669	5032	3.684
	1-Octanol, 2-nitro-	$C_8H_{17}NO_3$	175.23	30.365	5571	0.996
	Hydroxydehydrostevic acid	$C_{20}H_{30}O_3$	318.45	30.570	5612	11.338

hydroxydehydrostevic acid, phytol, and the triazolo-triazine derivative, indicating their main role in therapeutic activity. Phytol, a diterpene alcohol and precursor of vitamin E, has been shown to have antioxidant, anti-inflammatory, and neuroprotective properties, such as attenuation of oxidative stress and modulation of inflammatory cytokines[41,42].

The molecule is abundant, with a good docking profile with NFκB and α-synuclein, which makes it a promising complementary molecule to be used in PD therapy. The diterpenoid acid hydroxydehydrostevic acid was found to be very abundant and is a very promising lead compound from the molecular docking and ADMET analyses. The anti-inflammatory, antioxidant, and kinase-modulating properties of diterpenoid acids from other genera (Salvia and others) are of interest regarding neurodegeneration[43]. There is little direct evidence of hydroxydehydrostevic

Table 2. Binding free energies (ΔG) and inhibition constants (Ki)of ZO phytoconstituents to PD targets

Name of the Phytoconstituents	GSK-3β	KEAP1	LRRK2	NFκB	α-synuclein
	ΔG(kcal/mol) and Ki	ΔG(kcal/mol) and Ki	ΔG (kcal/mol) and Ki	ΔG (kcal/mol) and Ki	ΔG(kcal/mol) and Ki
Ethanediamide	-4.55(kcal/mol) and 463.19 μM	-4.18(kcal/mol) 864.46 μM	-4.58(kcal/mol) and 436.41 μM	-4.20(kcal/mol) and 835.14 μM	-3.64(kcal/mol) and 2.16 mM
DL-3-Aminobutyric acid	-3.32(kcal/mol) and 3.71 mM	-3.86(kcal/mol) and 1.49 mM	-5.21(kcal/mol) and 152.50 μM	-4.59(kcal/mol) and 433.77 μM	-5.27(kcal/mol) and 136.01 μM
L-Guanidinosuccinimide	-4.84(kcal/mol) and 283.23 μM	-5.78(kcal/mol) 57.65 μM	-6.29(kcal/mol) and 24.31 μM	-5.16(kcal/mol) and 166.30 μM	-6.25(kcal/mol) and 26.05 μM
L-[-]-4-Hydroxy-1-methylproline	-4.65(kcal/mol) and 388.58 μM	-4.82(kcal/mol) and 295.23 μM	-5.05(kcal/mol) and 199.99 μM	-4.72(kcal/mol) and 345.94 μM	-5.28(kcal/mol) and 134.79 μM
1,6;2,3-Dianhydro-4-deoxy-β-D-ribo-hexopyranose	-5.60(kcal/mol) and 78.57 μM	-5.01(kcal/mol) and 212.59 μM	-4.91(kcal/mol) and 250.54 μM	-4.94(kcal/mol) and 239.57 μM	-4.82(kcal/mol) and 295.09 μM
1,6;2,3-Dianhydro-4-deoxy-β-D-lyxo-hexopyranose	-3.74(kcal/mol) and 1.82 mM	-3.85(kcal/mol) and 1.51 mM	-4.22(kcal/mol) and 808.85 μM	-4.17(kcal/mol) and 879.21 μM	-4.31(kcal/mol) and 693.32 μM
Heptanoic acid, 2,4-dimethyl-, methyl ester, (2S,4R)-(+)-	-5.53(kcal/mol) and 88.91 μM	-4.83(kcal/mol) and 287.00 μM	-5.00(kcal/mol) and 214.52 μM	-4.74(kcal/mol) and 337.39 μM	-5.29(kcal/mol) and 132.19 μM
Phytol	-7.20(kcal/mol) and 5.25 μM	-6.18(kcal/mol) and 29.53 μM	-5.51(kcal/mol) and 92.04 μM	-7.39(kcal/mol) and 3.82 μM	-7.39(kcal/mol) and 3.81 μM
Pyruvic acid, 3-hexenyl ester	-5.50(kcal/mol) and 93.69 μM	-4.79(kcal/mol) and 306.04 μM	-1.16(kcal/mol) and 142.2 mM	-6.76(kcal/mol) and 11.08 μM	-6.81(kcal/mol) and 10.21 μM
3,7,11-Tridecatrienenitrile, 4,8,12-trimethyl-	-6.39(kcal/mol) and 20.84 μM	-5.62(kcal/mol) and 75.96 μM	-5.46(kcal/mol) and 100 μM	-6.85(kcal/mol) and 9.54 μM	-6.43(kcal/mol) and 19.46 μM
2,4-Dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c) triazine	-5.06(kcal/mol) and 196.65 μM	-5.58(kcal/mol) and 81.07 μM	-7.15(kcal/mol) and 5.71 μM	-5.79(kcal/mol) and 57.15 μM	-6.65(kcal/mol) and 13.42 μM
1-Octanol, 2-nitro-	-7.09(kcal/mol) and 6.40 μM	-5.93(kcal/mol) and 45.02 μM	-5.68(kcal/mol) and 3.60 μM	-6.97(kcal/mol) and 7.82 μM	-5.76(kcal/mol) and 60.28 μM
Hydroxydehydrostevic acid	-9.61(kcal/mol) and 89.97 nM	-10.58(kcal/mol) and 17.47 nM	-7.11(kcal/mol) and 6.19 μM	-9.71(kcal/mol) and 76.04 nM	-7.16(kcal/mol) and 5.64 μM
Levodopa	-7.33(kcal/mol) and 4.27 μM	-7.25(kcal/mol) and 4.87 μM	-7.04(kcal/mol) and 6.90 μM	-7.49(kcal/mol) and 3.22 μM	-7.47(kcal/mol) and 3.35 μM

acid in PD; however, its structural similarity with bioactive diterpenoid acids suggests its multi-target activity. In addition, the presence of 7-oxo-4,7-dihydro-triazolo(3,2-c)triazine, a heterocyclic derivative of triazine, is also known to be an inhibitor of kinases (LRRK2) and have anti inflammatory activities[44]. This limitation of GC–MS applies to volatile and thermally stable compounds only, and

Table 3. Molecular docking interactions of top-scoring phytoconstituents against the PD targets

Name of the Phytoconstituents	Target Proteins	Number of H-bonds	Conventional Type	Interacting Residues	Distance (Å)
Hydroxydehydrostevic acid	GSK-3β	5	Conventional H-bond	GLU B:597	2.00
			Conventional H-bond	ASN B:595	2.01
			Conventional H-bond	ARG B:596	2.15
			Conventional H-bond	LYS A:292	1.82
			Carbon H-bond	LYS A:292	2.91
			Alkyl	LYS A:292	5.07 and 5.12
			Alkyl	PHE B:567	4.74
			Pi- Alkyl	PHE B:567	5.35
			Alkyl	VAL A:263	3.15, 4.74 and 4.97
			Pi-Lone pair	PHE A:293	2.48
			Alkyl	PHE A:293	4.17
			Pi-Alkyl	PHE A:293	4.98 and 4.50
			Pi-Alkyl	TYR B:716	4.70
Hydroxydehydrostevic acid	KEAP1	4	Conventional H-bond	VAL A:463	1.96
			Conventional H-bond	ILE A:416	2.66
			Conventional H-bond	VAL A:606	1.99
			Alkyl	VAL A:606	4.85 and 5.38
			Conventional H-bond	GLY A:367	1.79
			Alkyl	ALA A:366	4.67
			Alkyl	ALA A:556	4.78
2,4-dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c) triazine	LRRK2	3	Conventional H-bond	VAL A:1373	3.36
			Carbon H-bond	ILE A:1371	3.62
			Carbon H-bond	VAL B:1373	3.34
			Attractive charge	ASP A:1372	3.15, 3.30, 3.45 and 3.89
			Attractive charge	ASP B:1372	2.87, 2.91, 3.33 and 3.55
Hydroxydehydrostevic acid	NFκB	2	Conventional H-bond	DG D:608	1.63
			Pi-Alkyl	DG D:608	5.06 and 5.31
			Conventional H-bond	DT D:610	2.26
			Unfavourable Donor	DA C:509	1.38
			Pi-Alkyl	TYR A:285	5.07
			Pi-Alkyl	TYR B:285	5.21
			Alkyl	LYS B:283	4.08
Phytol	α-synuclein	3	Conventional H-bond	GLU A:46	2.20
			Conventional H-bond	ARG A:67	2.84
			Carbon H-bond	SER A:338	3.63
			Pi-Alkyl	TYR A:342	4.44
			Alkyl	ARG A:345	3.68 and 5.07
			Alkyl	PRO A:155	4.90
			Pi-Sigma	TRP A:341	3.80
			Pi-Alkyl	TRP A:341	4.42 and 4.86
			Alkyl	ALA A:64	3.31, 4.41 and 4.73
			Alkyl	MET A:331	4.84
			Pi-Sigma	TRP A:63	3.75
			Pi-Alkyl	TRP A:63	4.07 and 5.45
			Pi-Alkyl	TYR A:156	4.13, 4.15 and 5.14

non-volatile and thermolabile compounds require LC-MS as a complementary future step.

3.3 Docking Analysis

Molecular docking assessed the binding affinities (ΔG) and predicted inhibition constants (Ki) of thirteen phytochemicals from ZO leaves extract against the PD targets (GSK-3β, KEAP1, LRRK2, NFκB and α-synuclein), as represented in Table 2. These data highlight Hydroxydehydrostevic acid, Phytol, and 2,4-dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c) triazine as promising multi-target inhibitors for neuroinflammatory and autophagic PD pathways, with Ki values superior to Levodopa. The key protein-ligand interactions, bond types, residues, and distances (Å) for top-ranking ligands were represented as data in Table 3 and Levodopa in Table 4, with the binding visualisation shown in Figure 2.

Hydroxydehydrostevic acid is the outstanding compound in Parkinson's target proteins (GSK-3β, KEAP1, LRRK2, NFκB and α-synuclein). This compound surpassed Levodopa in binding energy and inhibition constant (Ki), making it a promising multi-target lead for PD management. Hydroxydehydrostevic acid achieved better binding affinities against Levodopa with inflammatory protein targets (NFκB) and kinase (LRRK2 and KEAP1) pathways involved in PD pathogenesis. Its nanomolar Ki values (*e.g.*, 17.47 nM for KEAP1, 76.04 nM for NFκB) suggest high potency for mitigating α-synuclein aggregation, neuroinflammation, and mitochondrial dysfunction, which are characteristic of PD[45]. For example, *in silico* studies done with other plant diterpenoids, for example andrographolide derivatives and carnosic acid, typically showed binding free energies between −7.5 kcal/mol and −8.8 kcal/mol for GSK-3β. Hydroxydehydrostevic acid, on the other hand, had a significantly better docking score of −9.61 kcal/mol for GSK-3β, which means its specific peripheral hydroxyl and carboxylic acid substitutions make highly stabilised electrostatic anchors with core catalytic residues[46]. This data evidences Hydroxydehydrostevic from ZO may provide broad neuroprotective potential beyond Levodopa's dopaminergic action.

On the other hand, some phytochemicals were also observed to offer specific target benefits relative to Levodopa, providing them with possible combination therapy. Phytol demonstrates strong binding with NFκB (-7.39 kcal/mol, with Ki values of 3.82 μM), making it promising for inflammation and ubiquitination pathways. Finally, 2,4-dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c) triazine leads to LRRK2 inhibition (-7.15 kcal/mol, 5.71 μM Ki), positioning it as a kinase-focused candidate. Levodopa remains the gold standard for symptomatic Dopamine replacement, but it has limited therapeutic efficacy in multifactorial disease development, including proteopathy, neuroinflammation, and mitophagy[47].

3.4 *In Silico* ADMET Profiling and Evaluation

By using the SwissADME and ProTox-3.0 online tools, the parameters like pharmacokinetic, drug likeness, and toxicity profile of the compound are represented in Tables 5, 6, and 7, which are helpful in research for selecting suitable drug candidates and to identify and modify the compounds with adverse effects early in the stage of drug development.

The identified phytoconstituents were subsequently evaluated for their drug-like properties through pharmacokinetic profiling using SwissADME, which showed good properties for therapeutic use in Parkinson's disease. First of all, the best docking phytoconstituent, hydroxydehydrostevic acid, showed excellent drug-likeness with no violation of any of the five drug-likeness rules (Lipinski, Ghose, Veber, Egan and Muegge), and the highest bioavailability score of 0.85. This outstanding pharmacokinetic profile and BBB permeability make hydroxydehydrostevic acid the most promising lead compound for the development of anti-Parkinson drugs. Crossing the blood-brain barrier is very important for the treatment of PD, as the therapeutic agent should be able to penetrate dopaminergic neurons in the substantia nigra. Out of thirteen phytoconstituents, five were predicted to be BBB permeant and thus potential candidates for CNS disorders (Heptanoic acid; Pyruvic acid, 3-hexenyl ester; 3,7,11-Tridecatrienenitrile, 4,8,12-trimethyl-; 1-Octanol, 2-nitro; and Hydroxydehydrostevic acid).

The hydroxydehydrostevic acid was one of these compounds that had no rule violations, while also being BBB permeable. Metabolic interactions were also minimal, with most compounds having only weak CYP inhibitory profiles: phytol (CYP2C9 inhibitor) and hydroxydehydrostevic acid (CYP2C9 inhibitor) had CYP inhibitory activities, indicating lower drug-drug interaction risks. The P-gp efflux

Table 4. Protein-ligand interactions, bond types, residues, and distances (Å) for Levodopa docked to PD targets

Name of the Phytoconstituents	Target Proteins	Number of H-bonds	Type of Bonds	Interacting Residues	Distance (Å)
Levodopa	GSK-3β	9	Conventional H-bond	GLY A:262	2.20
			Conventional H-bond	TYR B:716	3.17
			Conventional H-bond	ILE B:728	2.61
			Conventional H-bond	SER A:215	2.23 and 2.61
			Conventional H-bond	TYR B:788	2.26 and 3.04
			Conventional H-bond	TYR A:288	2.22
			Conventional H-bond	ARG A:223	2.45
			Pi-cation	ARG A:223	3.90
			Carbon H-bond	GLY A:230	2.82
			Carbon H-bond	GLY B:730	3.15
			Pi-cation	ARG B:723	4.01
Levodopa	KEAP1	6	Conventional H-bond	VAL A:465	2.15
			Conventional H-bond	VAL A:512	2.70
			Conventional H-bond	VAL A:418	2.35
			Conventional H-bond	VAL A:467	2.66
			Conventional H-bond	VAL A:420	2.04
			Carbon H-bond	GLY A:419	3.23
			Alkyl	ILE A:559	4.89
			Pi-Alkyl	ALA A:466	4.15
Levodopa	LRRK2	4	Conventional H-bond	SER B:1366	2.15
			Conventional H-bond	GLN B:1365	1.66
			Conventional H-bond	SER B:1403	1.77
			Conventional H-bond	GLY A:1370	1.95
			Pi-Sigma	GLY A:1370	3.68
			Pi-Anion	ASP A:1375	3.84
Levodopa	NFκB	5	Conventional H-bond	LYS B:143	1.90 and 2.72
			Pi-Alkyl	LYS B:143	5.48
			Conventional H-bond	DA D:609	2.21
			Conventional H-bond	THR B:142	2.70
			Conventional H-bond	LYS B:144	2.06 and 2.20
			Conventional H-bond	DT D:611	2.82
			Pi-Anion	DT D:611	4.19
			Pi-Anion	DC C:513	4.70
Levodopa	α-synuclein	7	Conventional H-bond		
			Pi-Alkyl	ALA A:64	1.95
			Conventional H-bond	ALA A:64	4.21
			Conventional H-bond	LYS A:16	1.74
			Pi-Donor Hydrogen bond	TRP A:63	1.98
				TRP A:63	3.56
			Conventional H-bond	ASP A:66	2.98
			Conventional H-bond	ARG A:67	1.94 and 2.25
			Pi-Donor Hydrogen bond	TRP A:341	3.44
				TRP A:341	4.20
			Pi-Alkyl	TYR A:156	4.61
			Pi-Pi T Shaped		

and brain accumulation of hydroxydehydrostevic acid were also predicted, which may affect the efflux and brain accumulation of P-gp substrates. Only phytol was found to violate multiple drug-likeness rules (Lipinski, Ghose, Veber, and Egan violations). The bioavailability score for most compounds is within 0.50 and 0.55, indicating moderate oral bioavailability potential; hydroxydehydrostevic acid shows a higher

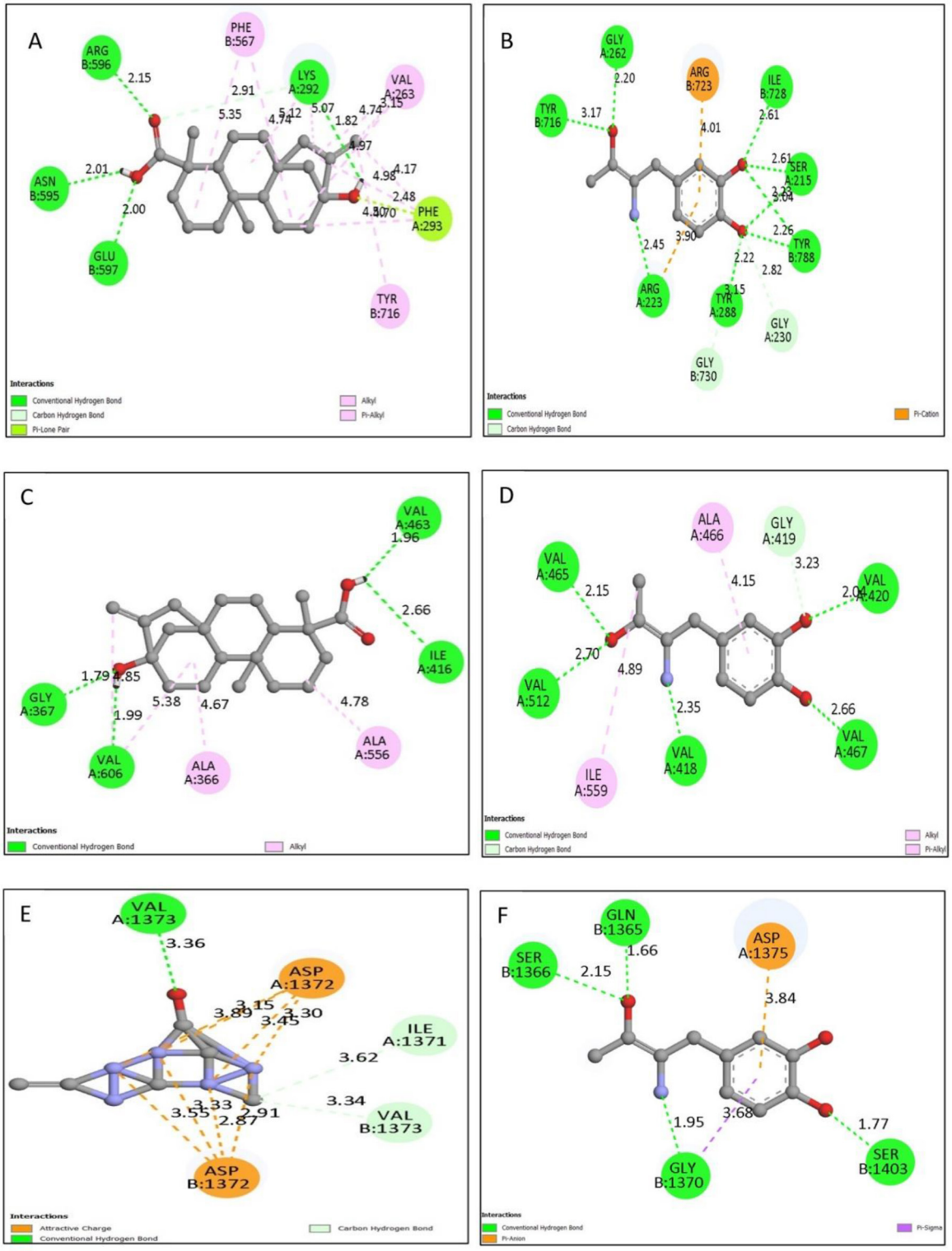


Figure 2. Visualisation of protein–ligand binding interactions generated with BIOVIA Discovery Studio Visualizer 2025. **(A).** GSK3β with Hydroxydehydrostevic acid; **(B).** GSK3β with Levodopa; **(C).** KEAP1 with Hydroxydehydrostevic acid; **(D).** KEAP1 with Levodopa; **(E).** LRRK2 with 2,4dimethyl7oxo4,7dihydrotriazolo(3,2-c)triazine; **(F).** LRRK2 with Levodopa; **(G).** NFκB with Hydroxydehydrostevic acid; **(H).** NFκB with Levodopa; **(I).** αsynuclein with Phytol; **(J).** αsynuclein with Levodopa.

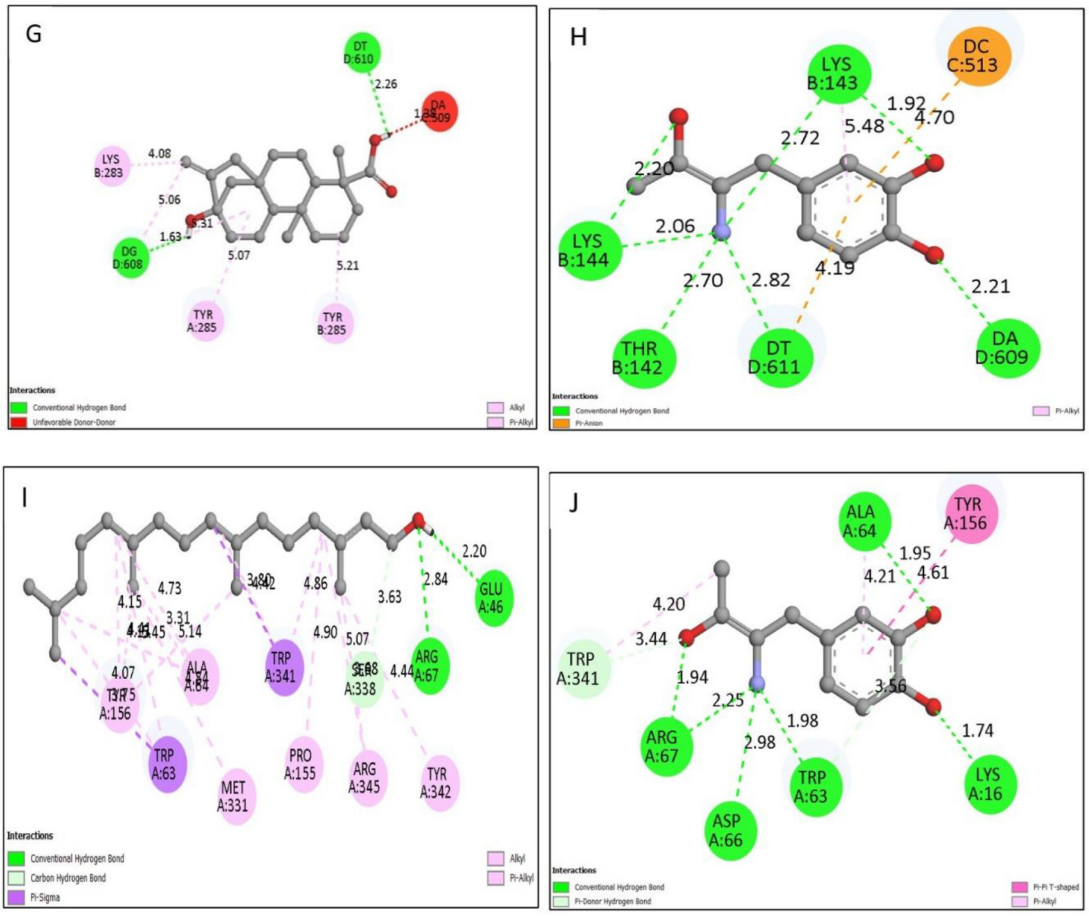


Figure 2. Continued.

Table 5. Pharmacokinetics descriptors for selected GC-MS phytoconstituents using SwissADME

Name of the Phytoconstituents	BBB Permeant	P-gp Substrate	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor
Ethanediamide	No	No	No	No	No	No	No
DL-3-Aminobutyric acid	No	No	No	No	No	No	No
L-Guanidinosuccinimide	No	No	No	No	No	No	No
L-[-]-4-Hydroxy-1-methylproline	No	No	No	No	No	No	No
1,6;2,3-Dianhydro-4-deoxy-β-D-ribo-hexopyranose	No	No	No	No	No	No	No
1,6;2,3-Dianhydro-4-deoxy-β-D-lyxo-hexopyranose	No	No	No	No	No	No	No
Heptanoic acid, 2,4-dimethyl-, methyl ester, (2S,4R)-(+)-	Yes	No	No	No	No	No	No
Phytol	No	Yes	No	No	Yes	No	No
Pyruvic acid, 3-hexenyl ester	Yes	No	No	No	No	No	No
3,7,11-Tridecatrienenitrile, 4,8,12-trimethyl-	Yes	No	No	No	No	No	No
2,4-Dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c)triazine	No	No	No	No	No	No	No
1-Octanol, 2-nitro-	Yes	No	No	No	No	No	No
Hydroxydehydrostevic acid	Yes	Yes	No	No	Yes	No	No

Table 6. Drug-likeness properties and bioavailability scores using SwissADME

Name of the Phytoconstituents	Lipinski Violations	Ghose Violations	Veber Violations	Egan Violations	Muegge Violations	Bioavailability Score
Ethanediamide	0	4	0	0	2	0.55
DL-3-Aminobutyric acid	0	3	0	0	3	0.55
L-Guanidinosuccinimide	0	4	0	0	1	0.55
L-[-]-4-Hydroxy-1-methylproline	0	3	0	0	2	0.55
1,6;2,3-Dianhydro-4-deoxy-β-D-ribo-hexopyranose	0	3	0	0	1	0.55
1,6;2,3-Dianhydro-4-deoxy-β-D-lyxo-hexopyranose	0	3	0	0	1	0.55
Heptanoic acid, 2,4-dimethyl-, methyl ester, (2S,4R)-(+)-	0	0	0	0	1	0.55
Phytol	1	1	1	1	2	0.55
Pyruvic acid, 3-hexenyl ester	0	0	0	0	1	0.55
3,7,11-Tridecatrienenitrile, 4,8,12-trimethyl-	0	0	0	0	1	0.55
2,4-Dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c)triazine	0	2	0	0	1	0.55
1-Octanol, 2-nitro-	0	0	0	0	1	0.55
Hydroxydehydrostevic acid	0	0	0	0	0	0.85

bioavailability score of 0.85, indicating high oral bioavailability potential. Hydroxydehydrostevic acid was found to have good docking affinity against the targets of PD, and toxicity prediction was carried out *in silico*, which flagged potential liabilities regarding organ-specific toxicities.

These can be overcome by a systemically delivered nanoformulation of phytochemicals, such as curcumin, quercetin or resveratrol, with only minimal side effects (*e.g.*, nanoformulation delivery by using PLGA or Eudragit polymeric nanoparticles)[48,49]. Second, ligand conjugation (*e.g.*, transferrin to cross the blood-brain barrier) offers the opportunity of selective brain targeting, which can reduce first-pass metabolism in the liver and cardiotoxicity[50]. Third, structure-activity relationship-based (SAR) structural optimisation can determine and modify toxicophores while maintaining the pharmacophore to minimise the carcinogenic potential[51]. Fourth, Design of Experiments (DoE) techniques can be used to optimise the dose needed to achieve the maximum degree of "neuroprotection" and minimum "toxicity"[52]. Also, lower doses of hydroxydehydrostevic acid, when used in combination with synergistic phytochemicals, result in less dose used, with no loss of efficacy. Further research will involve the formulation of PLGA-encapsulated hydroxydehydrostevic acid nanoparticles for targeted Parkinson's treatment, and the toxicological evaluation of these formulations and optimisation of the doses for *in vivo* applications.

4. Conclusion

In the analysed PD target proteins, hydroxydehydrostevic acid exhibits the highest value of binding energy and inhibition constant compared to Levodopa, and is the most potent multi-target inhibitor. Other compounds with target-specific properties, such as Phytol and 2,4-dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c) triazine, are included, with a focus on using combination therapy in the context of neuroinflammation and protein aggregation. However, the present study is only a preliminary study conducted *in silico*; further *in vitro* cell line studies and *in vivo* animal model studies are needed to be validated before moving into clinical applications. Specifically, neuronal cell models (*e.g.*, SH-SY5Y) could be used to test the neuroprotective activity, cytotoxicity, and antioxidant activity of the identified

Table 7. Toxicity predicted data of thirteen phytoconstituents by using the ProTox-3.0

Name of the Phytoconstituents	Hepato Toxicity	Neuro Toxicity	Nephro Toxicity	Respiratory Toxicity	Cardio Toxicity	Immuno Toxicity	Muta Genicity	Cyto Toxicity
Ethanediamide	No	No	No	No	No	No	No	No
DL-3-Aminobutyric acid	No	No	No	No	Yes	No	No	No
L-Guanidinosuccinimide	No	Yes	No	Yes	No	No	No	No
L-[-]-4-Hydroxy-1-methylproline	No	No	Yes	Yes	No	No	No	No
1,6;2,3-Dianhydro-4-deoxy-β-D-ribo-hexopyranose	No	No	No	No	Yes	No	Yes	No
1,6;2,3-Dianhydro-4-deoxy-β-D-lyxo-hexopyranose	No	No	No	No	Yes	No	Yes	No
Heptanoic acid, 2,4-dimethyl-, methyl ester, (2S,4R)-(+)-	No	No	No	No	No	No	No	No
Phytol	No	No	No	No	No	No	No	No
Pyruvic acid, 3-hexenyl ester	No	No	No	No	No	No	No	No
3,7,11-Tridecatrienenitrile, 4,8,12-trimethyl-	No	No	No	No	No	No	No	No
2,4-Dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c)triazine	No	Yes	No	Yes	No	No	No	No
1-Octanol, 2-nitro-	No	No	No	No	No	No	No	No
Hydroxydehydrostevic acid	Yes	No	No	Yes	Yes	Yes	Yes	No

lead phytoconstituents to confirm their efficacy *in vitro*. *In vivo* experiments in animal models of Parkinson's disease (MPTP-induced or 6-OHDA induced rat models) are then required to evaluate the following parameters: pharmacokinetics, brain penetration, behavioural improvements, and safety profiles. These experimental validations will provide further support to the validity of hydroxydehydrostevic acid as a lead compound, and its real therapeutic efficacy will be identified beyond computational predictions. The results will pave the way for hydroxydehydrostevic acid to be used in preclinical studies and, further, lead to a paradigm shift in the management of Parkinson's disease, beyond the control of the symptoms by Levodopa.

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