COMPUTATIONAL STUDIES APPLIED TO FLAVONOIDS OF GARCINIA MORELLA AGAINST PARKINSON'S DISEASES

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Abstract

Parkinson's is one of the major neurodegenerative disease of Alzheimer's, affects more than 6 million people around the word by causing motor dysfunction. In recent years natural products are widely used in the field of medicine. *Garcinia morella* is one of the major traditional plant which has antioxidant, antifungal, antiviral, hepatoprotective, anticancer, anti-inflammatory, antibacterial, and larvicidal active properties. Flavonoids are majorly reported for its capacity of crossing Blood Brain Barrier and slow down the progression of neurodegeneration. In the current study, we have screened for the flavonoids present in the *Gracinia morella* plant to analyse the activity of flavonoids against alpha synuclein using molecular docking technique. When compared to the standard drug levodopa, the Vitexin possess better interaction with alpha synuclein by forming 5 hydrogen bonds and highest binding affinity of -6.9 kcal/mol and exhibits as a potent lead molecule in Parkinson' s therapy.

Keywords: Parkinson's, alpha synuclein, flavonoids, phytobioactives, molecular docking, levodopa.

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1. Introduction

Different disorders of the central nervous system have been associated to the selective death of neurons, and the clinical symptoms differ depending on the neurons affected and the pattern of neuronal degeneration (L. Brichta et al., 2014). The progressive loss of neurons resulting in the muscle weakness and deterioration of the body's physiological functions. The postmitotic cells undergoes cell death, leading to cellular apoptosis signalling and oxidative stress (Lin and Beal et al., 2006). Additionally other pathological, gentical, biochemical and molecular factors affect the progression of the disease.

Parkinson's disease (PD) is the second most common neurological disease, after Alzheimer's. Parkinson's disease (PD) affects more than 6 million people around the world. Because it is an age-related neurodegenerative illness, and because life expectancy is increasing in most Western countries, the global population is expected to reach over 10 million by 2030 (Dorsey and Constrantinescu et al., 2007). A paucity of therapy alternatives for modifying the course of disease progression, along with an ageing population, foreshadows a growing financial burden on patients and payers (Kowal and Dall et al., 2013). The social impact on patients and caregivers is enormous, and the economic burden of PD in the United States is estimated to be $US14.4 billion per year (Kowal and Dall et al., 2013), with rising costs heavily weighted toward more advanced features of the disease, such as cognitive impairment, nonmotor symptoms, gait abnormalities, and falls. Symptomatic treatments for Parkinson's disease currently available primarily focus on dopaminergic signalling stimulation and can provide symptomatic relief for a limited time, but have little effect on nonmotor symptoms, and none have been shown to affect the progressive pathological and clinical decline. Recent studies have demonstrated the presence of proteins in the brains of the affected (involved in the process of NDDs), with modified physicochemical properties. The main neuropathological connections of motor damage in PD are increasing degradation of dopaminergic (DA) neurons, resulting in striatal dopamine depletion and formation of Lewy bodies in the substantia nigra (SN). Nonmotor symptoms include sadness, anxiety, emotional changes, cognitive impairment, sleep difficulty, and olfactory dysfunction (Kalia and Lang 2015). Resting tremor, rigidity, bradykinesia, gait difficulty, postural instability, and behavioural problems are among the symptoms (Sveinbjornsdottri 2016). Several investigations have found neurodegenerative variables including neuroinflammation (Shulman and Jager et al., 2011) as well as cytotoxic factors such IL1, NO, ROS, and TNF (Gao and Gao et al., 2017).

Carbidopa to replace dopamine, levodopa medicines, monoamine oxidase B inhibitors, dopamine agonists, catechol-o-methyltransferase inhibitors, anticholinergics, and amantadine are all used to treat Parkinson's disease (Diaz and Waters 2014). The most often used medicine to treat Parkinson's disease is levodopa (Miller and Hefter et al., 2004). These medications, on the other hand, have numerous side effects (Tamminga 2002) and frequently result in further difficulties, despite the fact that they do not cure or stop disease development. It's critical to find novel medicinal medicines with minimum negative effects.

Natural medicines, primarily chemicals derived from plants, have been increasingly used to treat PD in recent years since they are believed to have less adverse effects than synthetic medications (Shahpiri and Bahrmasoltani et al., 2016; Magalingam and Radhakrishnan et al., 2015). These advancements in PD treatment allow the disease to be treated effectively, resulting in symptom control and improved patient quality of life, frequently for decades after

the onset of the disease. The fruit-bearing tree *Garcinia morella* (Gaertn.) Desr., sometimes known as Indian gamboge, belongs to the Clusiaceae family and is a near relative of mangosteen (G. mangostana). It is a tropical evergreen tree that is native to the Indian subcontinent, Indochina, and Sri Lanka. It is found in abundance in India's Western Ghats and northeastern regions. The fruits of *Garcinia morella* is used in the treatment of dysentery, gastritis, etc. and is said to have anti-inflammatory properties. In addition to hydroxycitric acid and garcinol, kokam includes additional chemicals having antioxidant potential. Citric acid, malic acid, polyphenols, carbohydrates, anthocyanin pigments, and ascorbic acid are some of them (Mishra and Bapat et al., 2006).

Flavonoids, alkaloids, terpenoids, lignans, and phenols are examples of phytochemicals, which are a diverse group of naturally occurring bioactive molecules in plants. Phytochemicals are of great interest for treating NDDs because of their diverse chemical, biological, and molecular properties. Phytochemicals are attractive options for treating a variety of clinical illnesses by modulating several signal pathways and acting as antioxidants and anti-inflammatory agents (Uriarte and Calvo 2009), agents against cancer and neurological diseases (Hosseini and Ghorbani 2015), and antifungal agents (Windayani and Hakin et al., 2014). Recent studies on PD addressed that some active phytobioactive components has a neuroprotective activity. Flavonoids are the major chemical class in the plants, these flavonoids has a property of supressing lipid peroxidase, anti-inflammatory mediators and activation of antioxidant enzymes.

The current study is gared towards the effect of flavonoids present in the ***Garcinia morella*** for anti-Parkinson activity. Molecular Docking, in-silico technique is used to study the structural parameters involved.

2. Materials and Methods

Ligand Preparation

Based on the literature, we selected some of the flavonoid structure, known antioxidant action. The selective ligands were downloaded from NCBI, USA-PubChem chemistry database in 3-Dimensional form. The selective ligands were listed in **Table 1** and **Figure 1** (Murthy and Dalawai et al., 2020).

Protein Preparation

The 3-d structure of the protein 1XQ8 was retrieved from the Protein Data Bank (PDB). Further, the non-standard amino acids bound to the 1XQ8 protein were removed or modified using PYMOL software. The 3-D protein structure is represented in **Figure 2** (Dharmashekara and Pradeep et al., 2021).

Prediction of Binding Pockets

The binding pockets of 1XQ8 protein-ligand binding were predicted using the in-silico tool CASTp [**http://sts.bioe.uic.edu/in**, (accessed on 11 August 2021)] **Figure 3.**

Molecular Docking and Visualization

For a better understanding of the chemical interaction between 1XQ8 and the specific flavonoids, Pyrx version 0.8 software was employed. The preferred bonding residues achieved the highest orientations with the lowest [Kcal/mol] value for binding affinity. The BIOVIA Discovery studio Visualizer was used for the 2-D and 3-D visualization of the Docked molecules (Shreevatsa et al., 2021; Mishra et.al., 2006; (Shreevatsa B et al., 2022)

3. Results and Discussion

Table 2. Major flavonoid based phytobioactives from *Garcinia morella* with their chemical characteristics.

Sl. No	Compound Name	CID ID	Molecular Weight g/mol	Molecular Formula
1	Apigenin	5281675	**448.4**	$C_{21}H_{20}O_{11}$
2	Garcinia Biflavonoid 1	161087	**558.5**	$C_{30}H_{22}O_{11}$
3	Quercetin	5280343	**302.23**	$C_{15}H_{10}O_7$
4	Vitexin	5280441	432.4	$C_{21}H_{20}O_{10}$
5	Orientin			
6	Levodopa	**6047**	197.19	$C_9H_{11}NO_4$

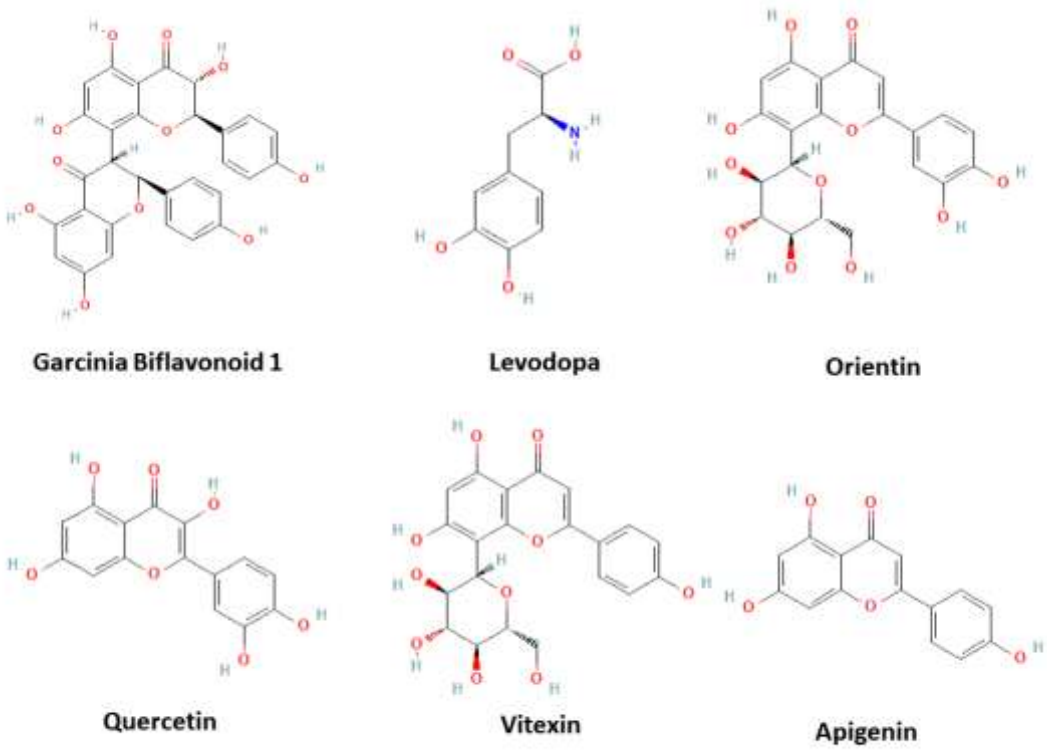


Figure 1. 2- Dimensional Structure of selective flavonoid based phytobioactives

Protein Preparation

The Protein Data Bank (PDB) was used to retrieve the 3-dimensional structure of the protein 1XQ8. Additionally, the 1XQ8 protein's non-standard amino acids were deleted or altered using the PYMOL program. The 3-D protein structure is represented in Figure 2 (Dharmashekara et al., 2021; Shreevatsa, B et.al., 2022).

Figure 2: 3-D Structure of Protein 1XQ8

Prediction of Binding Pockets

Using the in-silico technique CASTp, the binding pockets of 1XQ8 was predicted. [http://sts.bioe.uic.edu/in, [accessed on 11 August 2021]] Figure 3.

Figure 3: Predicted binding pockets of 1XQ8 protein

Protein-Ligand Interaction

The best-docked posture of the variety of sub-atomic docked compounds was considered, and the most reduced related constraining liking was marked, as shown in **Figure 4 and Table 2.** Using BIOVIA Discovery studio Visualizer software, these docked molecules were displayed and evaluated, with neighbouring tagged binding residues highlighted.

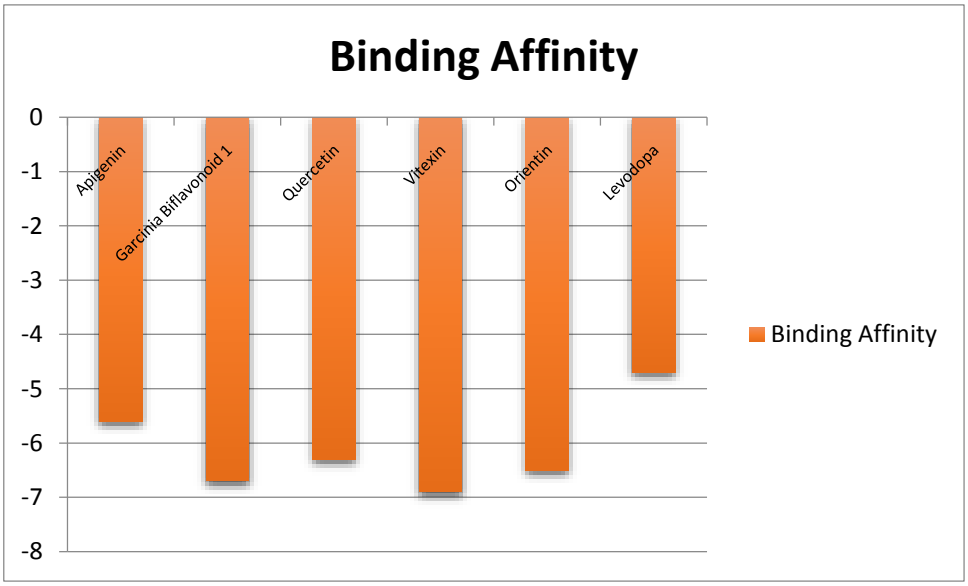


Figure 4. Histogram representing the binding energy value (ΔG) of selective flavonoid based phytobioactives against 1XQ8 protein.

Table 2. Binding affinity value and hydrogen bonding interaction of molecular docking analysis of selective flavonoid based phytobioactives against 1XQ8 Protein with respect to standard Levodopa molecule.

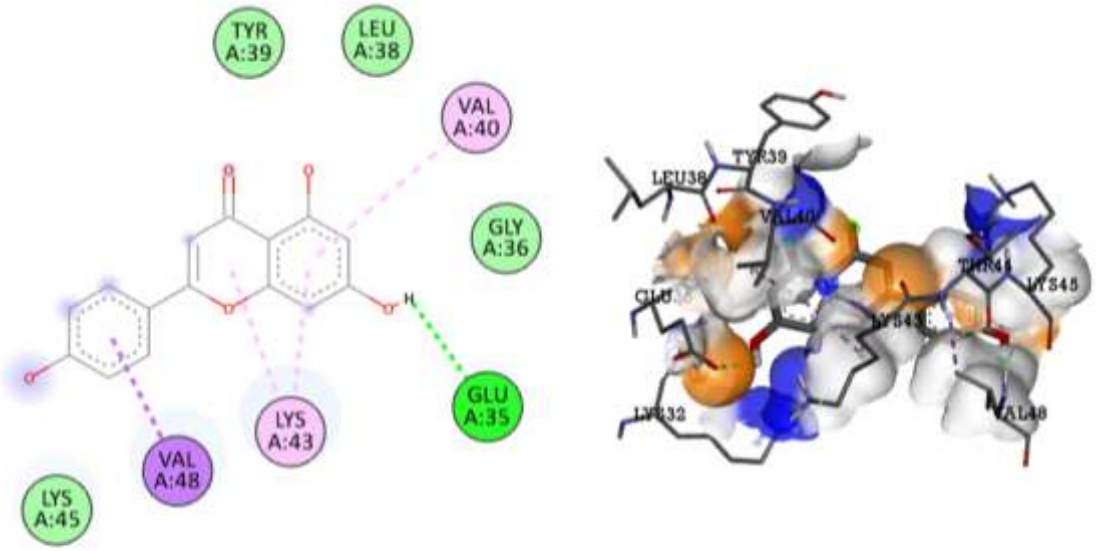
Sl. No	**Compound Name**	**Binding Affinity**	**Number of Hydrogen Bonds**	**Amino Acid Residues forming hydrogen bond**
1	Apigenin	-5.6	1	Glu 35
2	Garcinia Biflavonoid 1	-6.7	3	Val 40 Lys 45 Lys 32
3	Quercetin	-6.3	3	Glu 35 (2) Val 40
4	Vitexin	-6.9	5	Lys 32 Glu 35 (2) Leu 38 Gly 36
5	Orientin	-6.5	2	Ser 128 Gly 130
6	Levodopa	-4.7	5	Pro 128, Glu 126, Asp 135, Glu 130 Met 127

Molecular Docking interactions between 1XQ8 and Selective ligands.

Molecular docking is a widely utilised strategy in drug discovery that has little or no negative side effects. It's a two-step method that starts with ligand and target biomolecule geometrical optimization. The conformations in the receptor-identified active region are then ranked according to their score, which changes depending on the techniques employed to produce them from one programme to the next. As a result, **Figure 5D** vitexin interacts with 1XQ8 via hydrogen bond Gly 36, Leu 38, Glu 35 and 2 Lys 32 with other amino acids residues. These results implicate that vitexin binding with 1XQ8 is very strong compared to others.

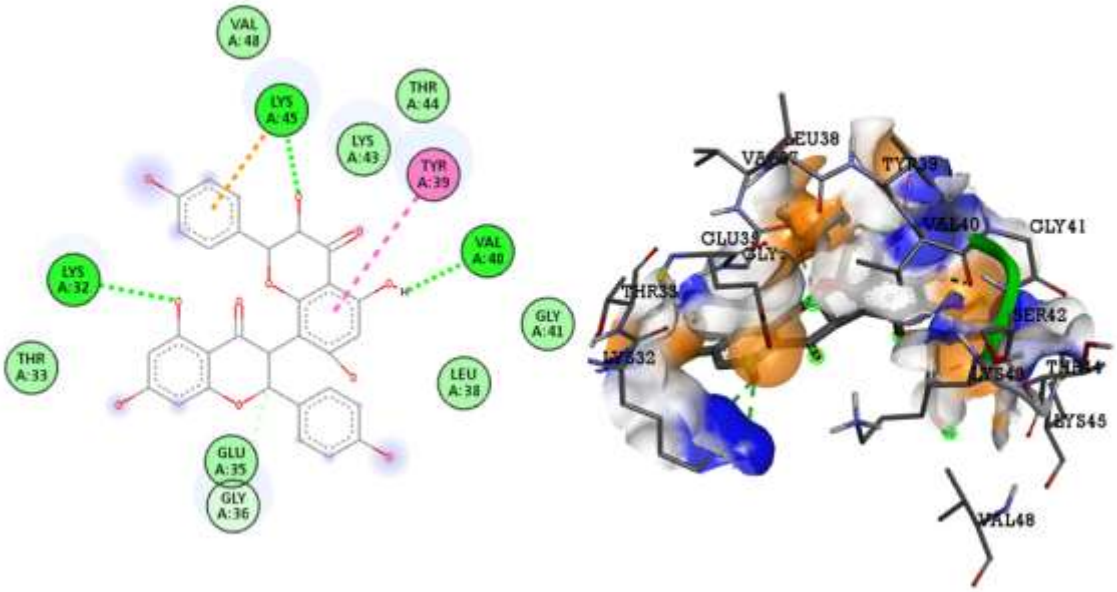
Figure 5. Shows the 2-D and 3-D visualised structures of selective flavonoid phytobioactives against 1XQ8 protein interactions, i.e., hydrogen bond and other lower interactions like vander waals force, π–π stacked, π–π T-shaped, π-alkyl and carbon hydrogen bond.

Figure 5A



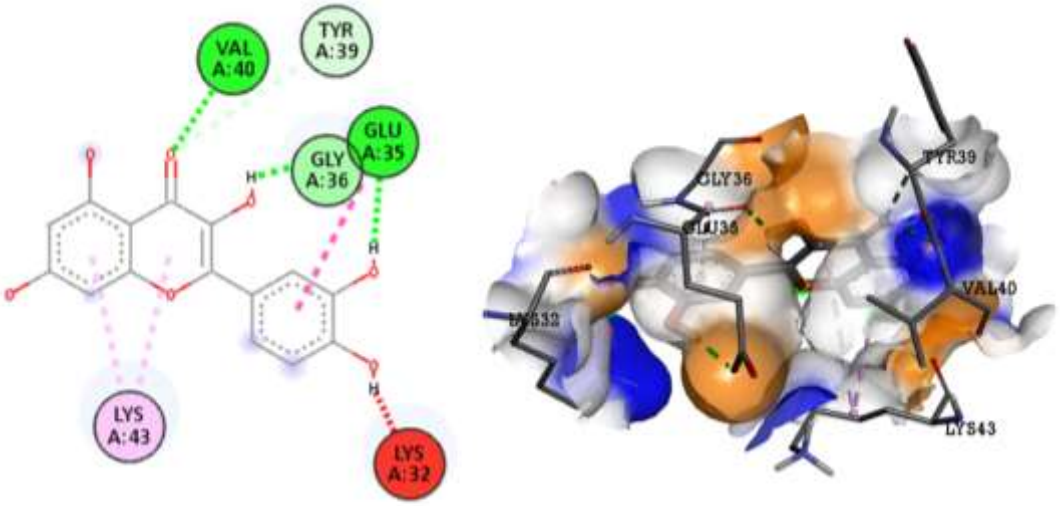
APIGENIN

Figure 5B



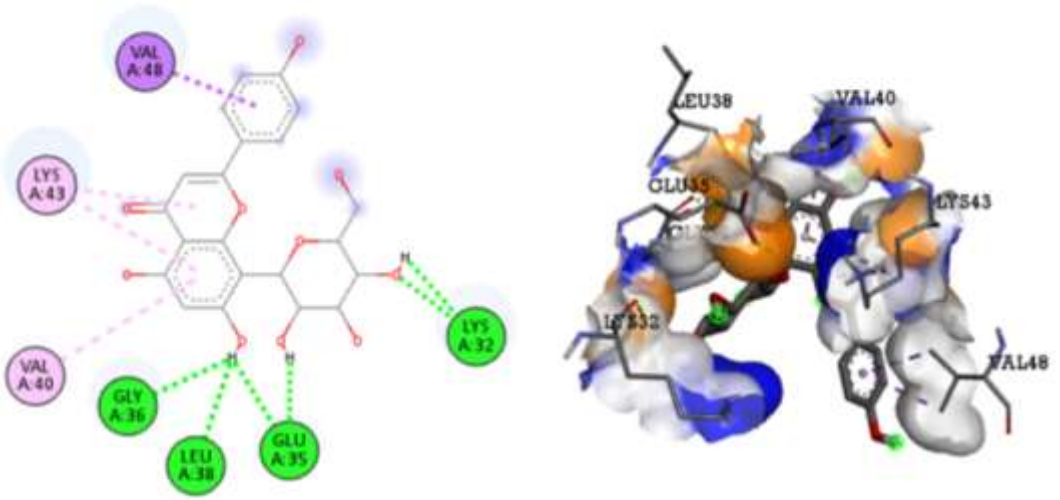
GARCINIA BIFLAVONOID 1

Figure 5C



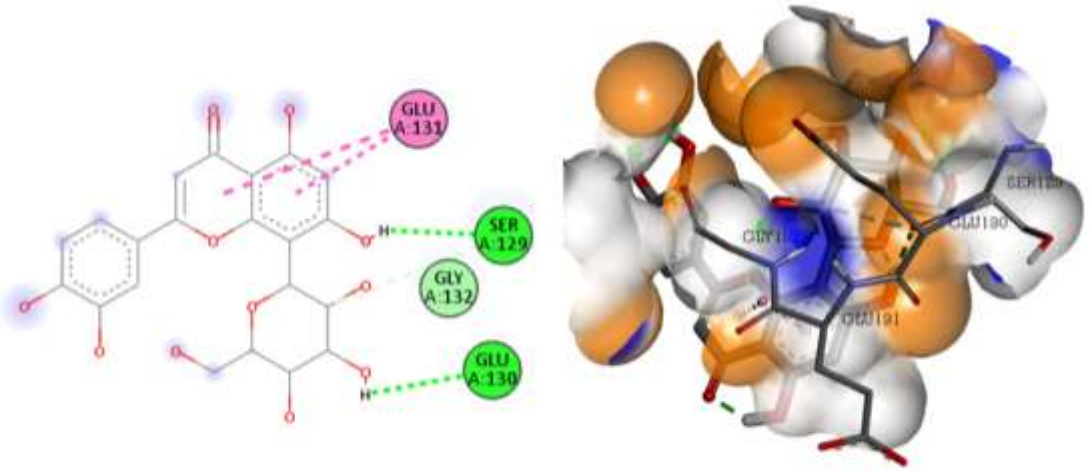
QUERCETIN

Figure 5D



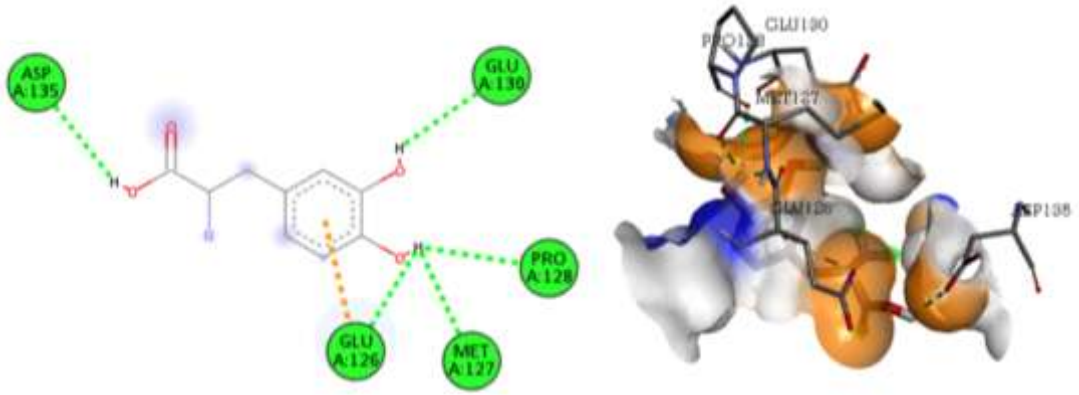
VITEXIN

Figure 5E



ORIENTIN

Figure 5F



LEVODOPA

Using the Molecular Docking (in silico) technique, we analyzed the interaction between the 1XQ8 protein and a selective flavonoid molecule. Levodopa is known as a potent (standard) molecule used in the reatment of Parkinson's. The **Levodopa (Figure 5F)** molecule (ligand) exhibits a binding affinity of −4.7 Kcal/mol by forming 5 hydrogen bonds with Pro 128, Glu 126, Asp 135, Glu 130 and Met 127 binding residuesof 1XQ8 protein, respectively. It also forms other interactions with the 1XQ8 protein. Similarly, **Apigenin** (**Figure 5**A) exhibits a binding affinity of −5.6 Kcal/mol and forms one hydrogen bond with Gly 35. **Garcinia Biflavonoid 1** (**Figure 5**B) shows the binding affinity of −6.7 Kcal/mol and forms three hydrogen bonds with the binding residues Val 40, Lys 45, and Lys 32. **Quercetin** (**Figure 5C**)

exhibits a binding affinity of −6.3 Kcal/mol and forms three hydrogen bonds with the binding residues 2 Glu 35 and Val 40. **Orientin (Figure 5E)** exhibits -6.5 of binding energy and by forms 2 hydrogen bonds with binding residues Ser 128 and Gly 130. The flavonoid Vitexin represents as a potent molecule when compared to the standard drug Levodopa.

4. Conclusion

We hereby conclude that from the current in-silico study, the flavonoid based phytobioactives of *Garcinia morella* possess potential neuroprotective activity by virture of binding to certain key target protein i.e., 1XQ8 of Parkinson's. Based our molecular docking studies the flavonoid Vitexin possess a better interaction with the target protein by forming 5 hydrogen bonds and other weak interactions too and forming highest binding affinity of -6.9 kcal/mol when compared to standard drug levodopa. In conclusion, the Indian Ayurvedic plants consist of different chemical class mainly flavonoids, which have a potency towards prevention of Parkinson's Disease.

Conflict of Interest

The authors declare there is no Conflict of interest.

5. References

a. Hosseini and A. Ghorbani, "Cancer therapy with phytochemicals: evidence from clinical studies," Avicenna Journal of Phytomedicine, vol. 5, no. 2, pp. 84–97, 2015.

b. A. Tamminga, "Partial dopamine agonists in the treatment of psychosis," Journal of Neural Transmission, vol. 109, no. 3, pp. 411–420, 2002.

2. Dharmashekara, C., Pradeep, S., Prasad, S. K., Jain, A. S., Syed, A., Prasad, K. S., Patil, S. S., Beelagi, M. S., Srinivasa, C., & Shivamallu, C. (2021). Virtual screening of potential phyto-candidates as therapeutic leads against SARS-CoV-2 infection. Environmental Challenges, 4, 100136. https://doi.org/10.1016/j.envc.2021.100136

3. Dorsey ER, Constantinescu R, Thompson JP, Biglan KM, Holloway RG, Kieburtz K, et al. Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. Neurology. 2007;68:384–6.

4. H. M. Gao, D. Tu, and Y. Gao, "Roles of microglia in inflammation-mediated neurodegeneration: models, mechanisms, and therapeutic interventions for Parkinson's disease," Advances in Neurotoxicology, vol. 1, pp. 185–209, 2017.

5. J. M. Shulman, P. L. de Jager, and M. B. Feany, "Parkinson's disease: genetics and pathogenesis," Annual Review of Pathology, vol. 6, no. 1, pp. 193–222, 2011.

6. K. B. Magalingam, A. K. Radhakrishnan, and N. Haleagrahara, "Protective mechanisms of flavonoids in Parkinson's disease," Oxidative Medicine and Cellular Longevity, vol. 2015, Article ID 314560, 14 pages, 2015

7. Kowal SL, Dall TM, Chakrabarti R, Storm MV, Jain A. The current and projected economic burden of Parkinson's disease in the United States. Mov Disord. 2013;28:311–8.

8. L. Brichta, P. Greengard, Molecular determinants of selective dopaminergic vulnerability in Parkinson's disease: an update, Front. Neuroanat. 8 (2014) 1e16, https://doi.org/10.3389/fnana.2014.00152

9. L. V. Kalia and A. E. Lang, "Parkinson's disease," Lancet, vol. 386, no. 9996, pp. 896–912, 2015.
10. M. T. Lin and M. F. Beal, "Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases," Nature, vol. 443, no. 7113, pp. 787–795, 2006.
11. Mishra A, Bapat MM, Tilak JC, Devasagayam T. Antioxidant activity of Garcinia indica (kokam) and its syrup. Curr Sci. 2006;91:90–3. [Google Scholar]
12. Murthy, H. N., Dalawai, D., Dewir, Y. H., & Ibrahim, A. (2020). Phytochemicals and Biological Activities of *Garcinia morella* (Gaertn.) Desr.: A Review. Molecules (Basel, Switzerland), 25(23), 5690. https://doi.org/10.3390/molecules25235690
13. N. L. Diaz and C. H. Waters, "Current strategies in the treatment of Parkinson's disease and a personalized approach to management," Expert Review of Neurotherapeutics, vol. 9, no. 12, pp. 1781–1789, 2014.
14. N. Windayani, Y. Rukayadi, E. H. Hakim, K. Ruslan, and Y. M. Syah, "Antifungal activity of lignans isolated from Phyllanthus myrtifolius Moon. against Fusarium oxysporum," Phytochemistry, vol. 12, pp. 33–39, 2014.
15. P. I. Uriarte and M. I. Calvo, "Phytochemical study and evaluation of antioxidant, neuroprotective and acetylcholinesterase inhibitor activities of Galeopsis ladanum L. extracts," Pharmacognosy Magazine, vol. 5, no. 20, p. 287, 2009.
16. S. Sveinbjornsdottir, "The clinical symptoms of Parkinson's disease," Journal of Neurochemistry, vol. 1, pp. 318–324, 2016.
17. Shreevatsa B, Dharmashekara C, Swamy VH, Gowda MV, Achar RR, Kameshwar VH, Thimmulappa RK, Syed A, Elgorban AM, Al-Rejaie SS, Ortega-Castro J, Frau J, Flores-Holguín N, Shivamallu C, Kollur SP, Glossman-Mitnik D. Virtual Screening for Potential Phytobioactives as Therapeutic Leads to Inhibit NQO1 for Selective Anticancer Therapy. Molecules. 2021; 26(22):6863. https://doi.org/10.3390/molecules26226863
18. Shreevatsa B, Dharmashekara C, Jain AS, Amachawadi R, Achar RR, Syed A, Shivamallu C, Kollur SP, Frau J, Flores-Holguín N, Glossman-Mitnik D. An insight into reactivity and bioactivity properties of quorum sensing peptides against PDE10A: a computational peptidology approach. J Mol Model. 2022 Jul 5;28(8):209. doi: 10.1007/s00894-022-05176-x. Erratum in: J Mol Model. 2022 Oct 8;28(11):347. PMID: 35789297.
19. T. Müller, H. Hefter, R. Hueber et al., "Is levodopa toxic?" Journal of Neurology, vol. 251, pp. 44–46, 2004.
20. Z. Shahpiri, R. Bahramsoltani, M. Hosein Farzaei, F. Farzaei, and R. Rahimi, "Phytochemicals as future drugs for Parkinson's disease: a comprehensive review," Reviews in the Neurosciences, vol. 27, no. 6, pp. 651–668, 2016.