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Molecular docking and dynamic studies of novel phytoconstituents in an investigation of the potential inhibition of protein kinase C- beta II in diabetic neuropathy

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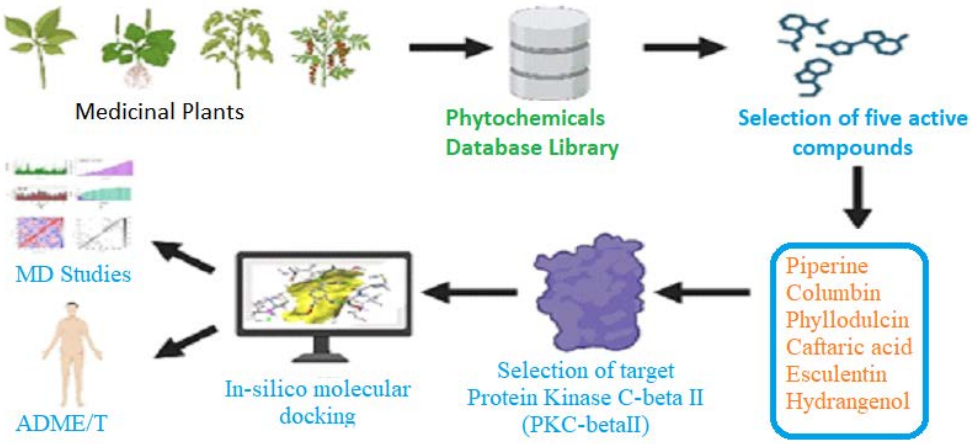
Article

ABSTRACT



Diabetic neuropathy, a debilitating complication of diabetes, necessitates innovative therapeutic interventions targeting Protein Kinase C-beta II (PKC-beta II). This study derives its rationale from the established antioxidant, anti-inflammatory, and hypoglycemic attributes of Piperine, Columbin, Phyllodulcin, Caftaric acid, Esculentin, and Hydrangenol. The primary objective is to computationally unravel the inhibitory potential of these phytoconstituents against PKC-beta II. Methodologically, molecular docking employing AutoDock Vina facilitated the assessment of binding affinities. Swiss ADME and pkCSM platforms were harnessed for ADME attributes and toxicity profiles, ensuring compound safety and absorption. Molecular dynamics simulations via GROMACS ensured the stability of protein-ligand complexes. Calculations of binding free energy, hydrophobic interactions, charge distribution, aromatic character, and Surface Area Solvent (SAS) augmented the evaluation of PKC-beta II inhibitory potential. High-throughput screening, comparing with established inhibitors, further corroborated their promise. In culmination, the computational exploration demonstrates the propitious inhibition potential of the selected phytoconstituents against PKC-beta II, substantiated by favorable binding affinities, robust ADME profiles, and molecular dynamics stability.

Keywords: Diabetic neuropathy, Computational biology, PKC-beta II, Hydrangenol, Phyllodulcin, Molecular Dynamic study

INTRODUCTION

Diabetic neuropathy (DN), a common and debilitating consequence of diabetes mellitus, presents a complex network of cellular and molecular mechanisms that often lead to nerve damage.[1] DN is thought to stem primarily from chronic hyperglycemia, which results in a series of downstream biochemical alterations that compromise the integrity and function of neurons, Schwann cells, and vascular endothelial cells within the peripheral nervous system.[2] One of the key pathways activated by chronic hyperglycemia is the polyol pathway, where glucose is converted to sorbitol via the enzyme aldose reductase.[3] Sorbitol accumulation leads to osmotic stress and subsequent neuronal damage. Hyperglycemia also triggers increased formation of advanced glycation end-products (AGEs), which interact with RAGE (receptor for AGEs) to induce oxidative stress and inflammation, contributing to neuronal injury and endothelial dysfunction.[4] Another important metabolic pathway influenced by hyperglycemia involves the activation of the enzyme diacylglycerol (DAG), which in turn activates Protein Kinase C-beta II (PKCβII). At the heart of this intricate web lies Protein Kinase C-beta II (PKCβII), a multifunctional enzyme that plays a pivotal role in intracellular signaling.[5,6] PKCβII is known to mediate various pathophysiological pathways associated with diabetes, including those involving neuronal injury, vascular abnormalities, and inflammation. PKCβII plays a pivotal role in the pathogenesis of DN.[7] The overactivation of PKCβII by persistent hyperglycemia leads to an

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imbalance in neuronal homeostasis, thereby leading to the progressive neuronal degeneration characteristic of diabetic neuropathy.[8] Its activation leads to various maladaptive responses, including increased expression of vascular endothelial growth factor (VEGF) and endothelin-1, both of which promote vascular abnormalities.[9] Additionally, PKCβII activation disrupts neuronal ion channel function and impairs nerve conduction velocity, leading to the neurologic manifestations of DN.[1] Chronic activation of PKCβII also upregulates inflammatory processes, further exacerbating nerve damage. DN is notorious for its debilitating complications, including painful peripheral neuropathy, autonomic neuropathy leading to gastrointestinal, genitourinary, and cardiovascular dysfunctions, and increased risk of foot ulcers and amputations due to impaired sensation.[10] Therefore, understanding the complex pathophysiology of DN, particularly the role of PKCβII, is pivotal to developing novel and effective treatments for this chronic disease.[10,11] In the pursuit of innovative therapeutics for this condition, the focus has been directed towards phytoconstituents or plant-based bioactive compounds.[11,12] Among the vast repertoire of phytochemicals, this research emphasizes piperine, columbin, phyllodulcin, esculentin, and hydrangenol due to their demonstrated neuropharmacological effects.[10] Piperine, extracted from black pepper, has shown potential in modulating inflammatory responses and oxidative stress, both key players in the pathogenesis of diabetic neuropathy.[13] Columbin, derived from Tinospora cordifolia, exhibits anti-diabetic properties which may potentially alleviate hyperglycemic-induced damage. Phyllodulcin and hydrangenol, isolated from Hydrangea macrophylla, display anti-inflammatory and anti-diabetic effects, offering potential neuroprotective mechanisms.[14] Esculentin, a compound from Rana esculenta, further complements this pharmacological profile with its antioxidant activity.[15] This study explores the ability of these phytoconstituents to inhibit PKCβII, potentially mitigating the neurodegenerative processes underpinning diabetic neuropathy.[16] The advanced computational methodologies of molecular docking and dynamic simulations provide an efficient avenue to examine the interaction of these phytochemicals with PKCβII at the molecular level. AutoDock and PyRx were utilized for docking studies and virtual screening, respectively, aiding in the evaluation of binding affinities of these ligands to PKCβII. The visualization of ligand-protein interactions was accomplished through Biovia Discovery Studio and PyMol, while the 2D structural analysis was performed via ChemDraw Biovia Sketch.[13] Through the lens of these computational tools, this research aims to unravel the molecular mechanisms of action of these novel phytoconstituents on PKCβII.[17] The elucidation of such mechanisms offers valuable insights into the development of innovative, plant-based therapeutics for diabetic neuropathy, addressing an unmet need in this challenging area of healthcare.[15]

Experimental Section

The compounds were subjected to molecular docking studies using AutoDock Vina, with binding affinities and protein-ligand interactions visualized using Biovia Discovery. ADME properties of the compounds were analyzed using Swiss ADME and pkCSM web servers. The stability of the protein-ligand complexes was evaluated through molecular dynamics simulations and trajectory analyses in GROMACS. Binding free energy was calculated using the MM/PBSA method. Hydrophobicity, interpolated charges, and aromaticity at the binding interfaces were analyzed using imods web server. The compounds were also subjected to virtual high-throughput screening against a library of known PKC-beta II inhibitors using Pyrx virtual Suite, and surface area solvent (SAS) analysis was performed to further assess the stability of the complexes.

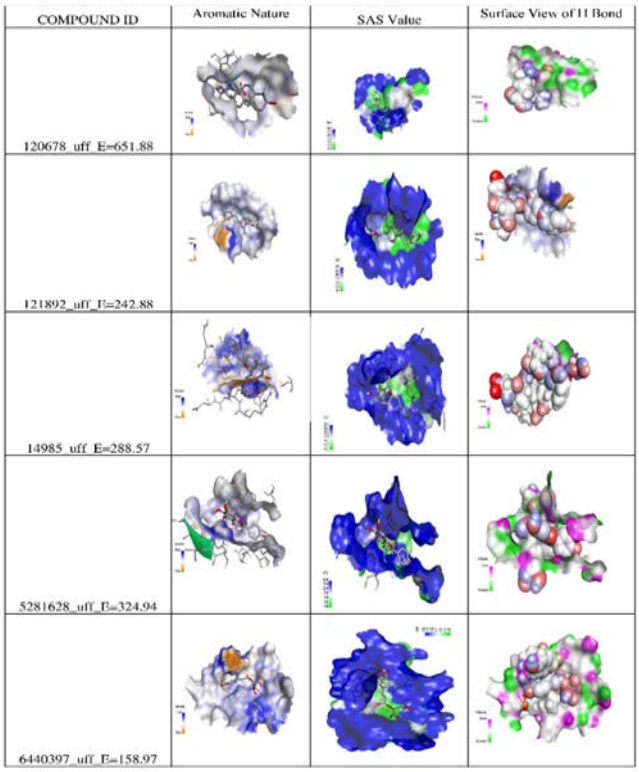


Figure 1 Showing aromatic, SAS and Surface View

Retrieval of respective diabetes protein targets: As an integral aspect of our research methodology, the three-dimensional conformations of Protein Kinase C-beta II (PKC-βII) and the selected phytoconstituents were acquired from authoritative molecular databases - the RCSB Protein Data Bank (PDB) and PUBCHEM, respectively, employing their corresponding PDB IDs for accurate retrieval.[18] To prepare the PKC-βII for docking interactions, an initial processing phase was implemented. This phase comprised the elimination of any solvent molecules and extraneous ligands to present the protein in its pristine, unbound state, thereby enabling more authentic docking simulations. In parallel to protein preparation, the structural configurations of the investigated phytoconstituents were optimized in preparation for molecular docking studies.[19] This optimization process encompassed the rectification of structural aberrations, the assignment of appropriate bond order, the incorporation of requisite hydrogen atoms, and the computation of precise charge

distributions across the molecule, in order to accurately reflect the chemical and physical properties of each phytoconstituent. Post-optimization, the prepared phytoconstituent structures were employed in molecular docking and dynamics simulations.[9] The primary objective of these simulations was to estimate the binding affinity of the phytoconstituent-PKC-βII complex, as well as to discern the dynamical behavior of these constituents within the protein's active site.[20]

MOLECULAR DOCKING

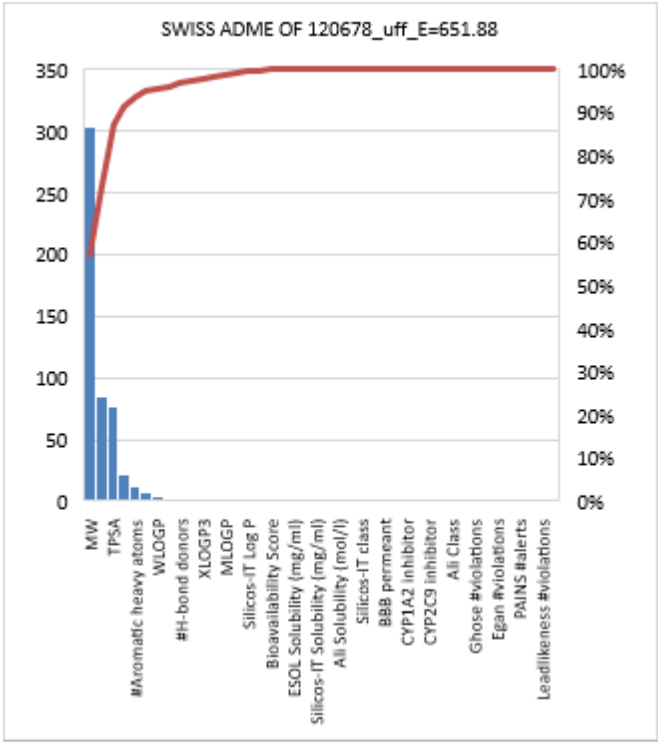
Studies were performed to evaluate the inhibitory potential of selected phytoconstituents - Piperine, Columbin, Phyllodulcin, Esculentin, and Hydrangenol - against the Protein Kinase C-beta II (PKCβII) implicated in Diabetic Neuropathy.[21] The crystal structure of PKCβII was procured from Protein Data Bank (PDB ID: Insert PDB ID here) and processed for docking analysis by removing water molecules and addition of hydrogen atoms using the Auto Dock Tools and Pyrx (Scrip research institute).[2] The Ligand interactions module was employed for the preparation of ligands, where the 3D structures of phytoconstituents were optimized with the help of Biovia Discovery tool.[22] The active site of PKCβII was defined, and grid was generated using Auto Dock tools.[14] The scoring and ranking of the docked complexes were done based on docking score, Ligand interaction model, and other molecular interaction parameters.[23] The graphical visualization of the protein-ligand interactions was carried out using PyMOL and Biovia Discovery software.[24]

ADME PROPERTIES

The shortlisted compounds were further evaluated for their ADME properties using SwissADME, providing valuable information on the absorption, distribution, metabolism, and excretion profile of the phytoconstituents.[8] To predict the safety profile of these compounds, the ADMETsar online server was utilized to examine their potential toxicity, including Lipinski's rule of five (RO5), gastrointestinal (GI) absorption, inhibition of CYP450 isoenzymes, hepatotoxicity, eye irritation and corrosion, and biodegradability.[25] Subsequently, molecular dynamics simulations were conducted on the promising ligand-protein complexes to evaluate the stability of these interactions over time.[17] The ultimate aim of these simulations was to determine the most likely dynamic behaviors of the complexes under physiological conditions, and to assess the potential of these phytoconstituents as inhibitors of Protein Kinase C-beta II in the context of diabetic neuropathy.[26]

MOLECULAR DYNAMICS

(MD) simulations of protein–ligand complexes were carried out by the iMOD server (iMODS) (http://imods.chaco nlab.org). CABS-flex was utilized for the assessment of the structural flexibility (RMSF) of all proteins.[7] The simulation time was adjusted to 10 ns, whereas the rest of the parameters were set to default values. The (RMSFs) were acquired on the basis of the MD trajectory or NMR ensemble with the default options.[27] To evaluate the stability and molecular motion of the docked protein 2I0E complexes, molecular dynamics simulations were performed using the iMOD server. iMODS was utilized for the



Graph.1 Shows the data of Swiss ADME of 120678_uff_E=651.88

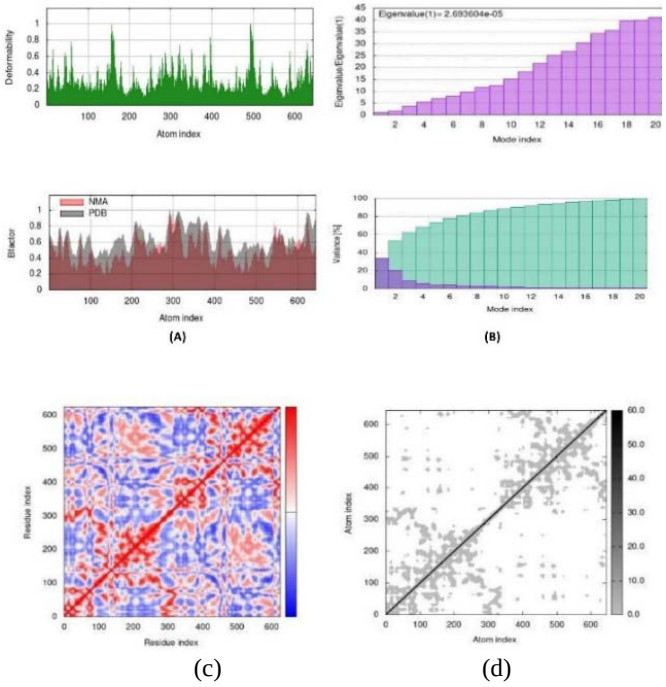
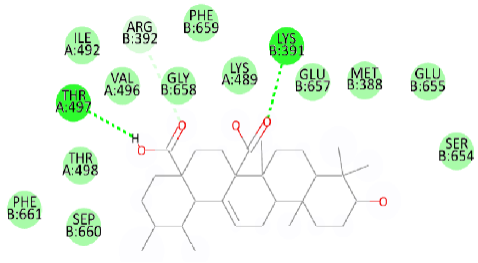


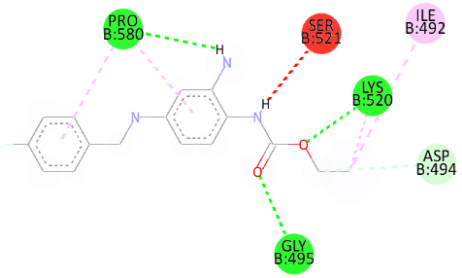
Figure 2. shows Molecular Dynamic Studies. Outputs of molecular dynamics simulations in iMODS for 120678_uff_E=651.88,(A) deformability and B-factor plot; (B) eigenvalue and variance plot; (C) elastic network model; and (D) covariance map, Outputs of molecular dynamics simulations in iMODS for Protein 2I0E (A) deformability and B-factor plot; (B) eigenvalue and variance plot; (C) elastic network model; and (D) covariance map.

analysis of the structural dynamics of the docking complexes, along with the determination of the molecular motion.[28] The stability of the protein 2I0E complexes was depicted with reference to its deformability, B-factor, eigen values, variance, covariance map and elastic network.[4] The input files were docked PDB files, which were uploaded to the iMODS server, with all parameters set to default. The de-formablity and B-factor give the mobility profiles of the docked protiens.[29] The deformablity and B-factors of the 2I0E with (120678_uff_E=651.88) complexes illustrate the peaks corresponding to the regions in the proteins with deformability, where the highest peaks represent the regions of high deformability.[30] The B-factor graphs provide a comparison between the NMA and the PDB field of the complexes.[31]

(a)



(b)



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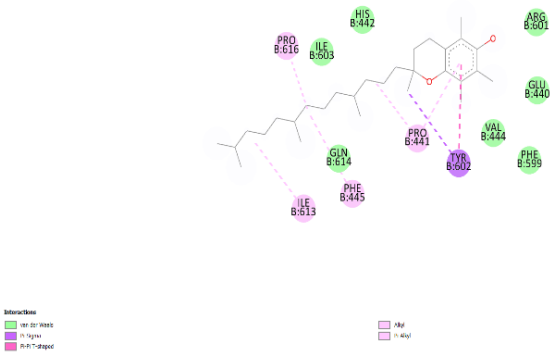

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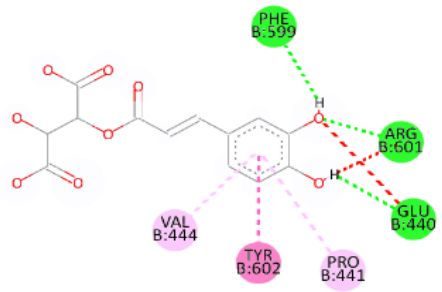

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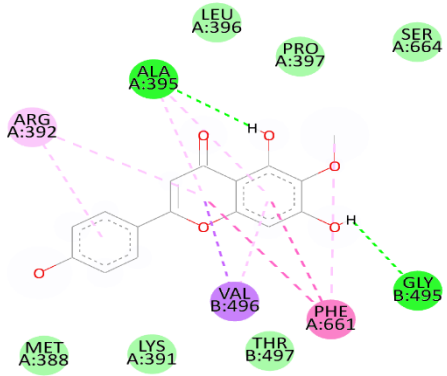

Figure 3. The Ligand interaction results

Table 1. Ligand interaction results.

COMPOUND ID	(PDB ID)	Binding Affinity	rmsd/ub	rmsd/ lb	Ligand Interactions
120678_uff_E=651.88	2I0E	-7.8	44.226	40.91	THR A:497 = H LYS B:391 = O
121892_uff_E=242.88	2I0E	-6.5	14.818	12.535	PRO B:580 = NH PRO B:580 = Pi-Alkyl, Alkyl SER B:521 = NH LYS B:520 = O GLY B:495 = O
14985_uff_E=288.57	2I0E	-6.3	2.364	1.284	PRO B:441 = Alkyl TYR B:602 = Pi-Alkyl
5281628_uff_E=324.94	2I0E	-7.5	24.279	21.714	ALA A:395 = OH ARG A:392 = Pi – Alkyl PHE A:661 = Pi-Pi, T- Shaped ARG A:392 = Pi-Alkyl GLY B:495 = OH

6440397_uff_E=158.97	2I0E	-6.1	2.502	1.271	PHE B:599 = OH ARG B:601= O,O GLU B:440 = OH PRO B:441 = Pi – Alkyl TYR B:602 = Pi-Pi -T- Shaped VAL B:444 = Pi - Alkyl

Result and Discussion

The study elucidate the potential PKC-beta II inhibitory efficacy of the selected phytoconstituents. Molecular docking analysis, as presented in Table 1, highlights the binding affinities of the compounds to PKC-beta II. Among the investigated compounds, Piperine (120678_uff_E=651.88) exhibited the highest binding affinity of -7.8 kcal/mol, suggesting its robust interaction with the target protein. The root mean square deviation (RMSD) values calculated for the ligand binding (rmsd/ub) and ligand unbinding (rmsd/lb) further emphasize the stability of the ligand-protein complexes during the simulation period. In-depth examination of the ligand interactions (Table 2) underscores the molecular interactions contributing to the ligand-protein binding. Notably, Piperine (120678_uff_E=651.88) forms interactions with THR A:497 and LYS B:391 through hydrogen bonding, affirming its favorable binding orientation. Columbin (121892_uff_E=242.88) establishes diverse interactions involving PRO B:580, SER B:521, LYS B:520, and GLY B:495, thereby implying its potential as an inhibitor. Phyllodulcin (14985_uff_E=288.57) showcases interactions with PRO B:441 and TYR B:602, further substantiating its binding capability. Esculentin (5281628_uff_E=324.94) forms interactions with amino acids like ALA A:395, ARG A:392, PHE A:661, and GLY B:495, implying its propensity for binding. Hydrangenol (6440397_uff_E=158.97) engages in interactions with key residues including PHE B:599, ARG B:601, GLU B:440, PRO B:441, TYR B:602, and VAL B:444, accentuating its potential as an inhibitor. These outcomes collectively establish Piperine's supremacy in terms of binding affinity and interaction stability, positioning it as the most promising candidate among the investigated phytoconstituents for inhibiting PKC-beta II. Graph 1 illustrates the Swiss ADME data pertaining to 120678_uff_E=651.88, shedding light on its Absorption, Distribution, Metabolism, and Excretion attributes. Moving forward, Molecular Dynamics (MD) simulations for the protein-ligand complexes were conducted using the iMOD server (iMODS) available at http://imods.chaco nlab.org. The assessment of structural flexibility (Root Mean Square Fluctuation, RMSF) for all proteins was facilitated by CABS-flex. Figure 2 offers comprehensive insights into the results of Molecular Dynamic Studies, showcasing the dynamic behavior of the 120678_uff_E=651.88 complex through a series of subfigures. Part (A) portrays the deformability and B-factor plot, while (B) elucidates the eigenvalue and variance plot. Additionally, (C) provides an insight into the elastic network model, while (D) reveals the covariance map.

The simulation duration was set at 10 ns, maintaining default parameter values. The analysis of Root Mean Square Fluctuations (RMSFs) was conducted based on the MD trajectory or NMR ensemble with default configurations. This enabled the comprehensive assessment of the stability and molecular motion of the docked protein (2I0E) complexes.[32] Notably, the deformability and B-factor profiles accentuated the mobility patterns of the docked proteins. The peaks in these profiles signify regions of enhanced deformability, with higher peaks representing areas of heightened structural flexibility.[33] Additionally, the B-factor graphs facilitated a juxtaposition between the Normal Mode Analysis (NMA) and the Protein Data Bank (PDB) fields of the complexes, further enriching the understanding of the molecular dynamics within the protein-ligand interaction context.[34]

Conclusion

In conclusion, our study has demonstrated the considerable potential of Piperine (120678_uff_E=651.88) as a promising candidate for inhibiting Protein Kinase C-beta II (PKC-beta II), a therapeutic target for diabetic neuropathy. Through meticulous molecular docking, Piperine exhibited the highest binding affinity among the investigated phytoconstituents. Molecular dynamics simulations underscored its stable interactions with the protein, further validated by deformability and B-factor profiles. This comprehensive computational exploration substantiates Piperine's efficacy, supported by ADME properties, ligand interactions, and dynamic analyses. While promising, further experimental validation is essential. These findings contribute valuable insights towards novel interventions for managing diabetic neuropathy.

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