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IN SILICO INVESTIGATION OF PIPER CHABA HUNTER PHYTOCHEMICALS FOR MODULATING KEY PROTEIN TARGETS IN ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer's disease (AD) is a relentless neurodegenerative condition that slowly erodes memory and cognitive function, leaving a profound impact on patients and their families. The complexity of its causes, rooted in a tangle of genetic and environmental factors, has so far stymied efforts to find a cure. Current treatments offer only temporary relief from symptoms, creating a pressing need for new therapeutic strategies that can tackle the disease's underlying mechanisms. In the search for new solutions, we turn to nature's vast apothecary. Medicinal plants like Piper chaba Hunter, a traditional spice and remedy, offer a rich source of bioactive compounds and have long been noted for their anti-inflammatory properties—a key feature in the fight against AD. This study embarks on a computational journey to unlock the therapeutic secrets of Piper chaba. Using a powerful suite of in silico tools, we aimed to identify its key bioactive compounds and map out how they might work together to combat the molecular machinery of Alzheimer's disease. Our investigation began by compiling a library of phytochemicals from Piper chaba and putting them through a rigorous virtual screening to assess their drug-like potential. We predicted their protein targets and cross-referenced them with AD-related gene databases to build a network of interactions, revealing the most influential protein "hubs." We then used high-precision molecular docking, extended 100-nanosecond molecular dynamics (MD) simulations, and Density Functional Theory (DFT) to investigate the binding and stability of these compounds with their targets. Our results show that compounds from Piper chaba, particularly piperine, have excellent drug-like qualities and are predicted to cross the blood-brain barrier. Our network analysis pointed to three key protein hubs—PTGS2 (COX-2), PLA2G4A, and CYP2C19—that are central to the neuroinflammatory and metabolic chaos of AD. The docking and MD simulations confirmed that lead compounds form strong, stable complexes with these proteins. This computational exploration strongly suggests that the humble Piper chaba plant holds a sophisticated arsenal of compounds that can wage a multi-pronged assault on Alzheimer's disease, representing promising candidates for a new generation of multi-target AD therapies.

Keywords: Alzheimer's Disease; Piper chaba; Network Pharmacology; Molecular Docking; Molecular Dynamics; Piperine; Neuroinflammation; In Silico Drug Discovery; PTGS2; PLA2G4A; Computational Chemistry.

INTRODUCTION

Alzheimer's disease (AD) is more than a clinical diagnosis; it is a slow unraveling of the self. It represents one of the most profound challenges to modern medicine and a growing global health crisis that casts a long shadow over our aging population [3]. For those affected, the journey begins with small, often dismissed, lapses in memory and culminates in a devastating loss of identity, connection, and the ability to navigate the simplest aspects of daily life [5]. This heartbreaking decline is the surface manifestation of a silent, complex war being waged within the brain.

For decades, the scientific community has focused on two primary culprits in this war: amyloid-beta (Aβ) peptides, which clump together to form toxic plaques between neurons, and hyperphosphorylated tau proteins, which form tangles inside them [1, 34]. The "amyloid cascade hypothesis" long held that these plaques were the first shot fired, triggering a downstream cascade of events leading to widespread neuronal death [7]. Yet, the consistent failure of drugs designed to target only amyloid has forced a broader, more humbling realization: Alzheimer's is not a simple linear problem. It is a multifactorial disease, a complex storm where Aβ and tau pathologies are whipped together with chronic

neuroinflammation, failing cellular power plants (mitochondria), rampant oxidative stress, and a breakdown of the brain's waste disposal systems [2, 4, 46]. In this storm, the brain's own immune cells, microglia and astrocytes, can turn from protectors to aggressors, creating a self-perpetuating cycle of damage [2]. This intricate web of pathology explains why a single "magic bullet" has remained elusive and why a new approach is desperately needed [47].

The current arsenal of approved AD treatments is strikingly limited. Available drugs provide only a temporary reprieve from symptoms for some, doing little to halt the relentless march of the underlying disease [6]. This therapeutic void has created an urgent imperative to find new strategies that can modify the course of the disease itself. In response, the paradigm of drug discovery is undergoing a fundamental shift. The old model of "one target, one drug" is giving way to a more sophisticated strategy: the development of multi-target-directed ligands (MTDLs) [23, 47]. The goal is no longer to find a single key for a single lock, but to design a master key or a set of synergistic tools that can engage multiple pathological checkpoints at once. This approach, which mirrors the complexity of the disease itself, is believed to hold the promise of greater efficacy and a more durable therapeutic response.

Nature has always been our most gifted chemist. For millennia, traditional systems of medicine have harnessed the power of plants to treat human ailments, including those affecting the mind and memory [9, 15]. Modern science is now beginning to understand the wisdom behind these ancient practices. Plants produce a dazzling array of phytochemicals—alkaloids, flavonoids, terpenoids, and more—that have been honed by evolution to interact with biological systems [8, 72]. Many of these compounds have been shown to possess potent antioxidant, anti-inflammatory, and neuroprotective properties directly relevant to the fight against AD [10, 11, 13, 71, 73]. Furthermore, the natural synergy found within a single plant extract, where multiple compounds work in concert, is the perfect embodiment of the multi-target therapeutic strategy that AD demands [12, 14].

Among the vast botanical kingdom, the Piper genus is a standout, known for its rich and pungent chemistry. Piper chaba Hunter, a vine native to Southeast Asia, is a beloved spice and traditional remedy in regions like Bangladesh [16]. While its cousin, Piper longum, has received more attention for its neuroactive compounds [17, 18], P. chaba is now emerging from the shadows. Previous studies have highlighted its impressive anti-inflammatory, analgesic, and immune-modulating effects [19]. Since neuroinflammation is a raging fire that fuels the progression of AD [2], the potent anti-inflammatory nature of P. chaba makes it a compelling candidate for a "neuro-soothing" agent. We hypothesize that its phytochemicals could act as a fire brigade, quenching the inflammatory cascade in the brain and protecting vulnerable neurons from harm.

This study was born from a desire to connect the dots between the traditional wisdom surrounding Piper chaba and the cutting-edge needs of modern neurotherapeutics. By weaving together advanced computational techniques, we aim to build a strong, data-driven case for the anti-Alzheimer's potential of this remarkable plant.

2. METHODS

Our investigation began with a meticulous search of the scientific literature to build a comprehensive library of compounds previously isolated from Piper chaba Hunter. We systematically queried major databases like PubMed, Scopus, and Google Scholar to find studies that had analyzed the plant's chemical makeup. For each identified phytochemical, we verified its structure and retrieved its 3D coordinates from the authoritative PubChem database [29]. Using the Avogadro molecular editor [41], each structure was then carefully prepared for the demanding computational analyses to follow, ensuring all atoms and bonds were correctly represented—a critical quality control step for any in silico study. This process effectively created a virtual inventory of the plant's chemical arsenal.

A promising compound is useless if it cannot get to where it needs to go in the body or is quickly eliminated. To address this, we subjected our phytochemical library to a rigorous ADME (Absorption, Distribution, Metabolism, and Excretion) screening using the SwissADME web server [28]. This virtual trial assessed several critical parameters, including drug-likeness (e.g., Lipinski's "rule of five"), pharmacokinetics (GI absorption and blood-brain barrier permeability), and medicinal chemistry friendliness (screening for toxic motifs like PAINS). This filtering process allowed us to separate the most promising, drug-like candidates from the rest.

With our list of promising compounds, the next question was: what do they actually do in the body? To answer this, we used a dual-strategy approach to predict their protein targets, using both SwissTargetPrediction [31] and the Similarity Ensemble Approach (SEA) [30]. After compiling a master list of potential protein targets, we performed the crucial step of linking them to Alzheimer's disease by cross-referencing our list against multiple gold-standard disease databases (OMIM [32], DisGeNET [33], and AlzGene). This allowed us to create a final, high-confidence list of "AD-relevant targets."

To truly understand the data, we used Cytoscape software to build a visual map—a "compound-target-disease" network. To delve deeper, we constructed a Protein-Protein Interaction (PPI) network using the STRING database [24], which reveals how our target proteins "talk" to each other. By analyzing the topology of this network, we could identify the "hub proteins"—the most highly connected and influential players in the disease process [21].

Here, we moved from the network level to the atomic level. Using the PyRx virtual screening tool [35] with the AutoDock Vina engine, we performed molecular docking simulations. We took the 3D structures of our hub proteins (prepared with YASARA [36]) and tried to fit each of our lead compounds into their active sites, calculating a "binding energy" score for each fit.

Docking provides a static snapshot, but biology is dynamic. To see how these interactions hold up, we ran all-atom MD simulations for an extended 100-nanosecond period. Using YASARA and the AMBER force field [36, 37], we placed each top-ranked protein-ligand complex in a simulated physiological environment [38, 39, 40]. By analyzing the simulation trajectory, we could measure stability (RMSD), flexibility (RMSF), and compactness (Rg). To add another layer of quantitative rigor, we used the MM/PBSA method to calculate a more accurate binding free energy from the stable portion of our MD trajectories.

For our final analysis, we delved into the subatomic world using Density Functional Theory (DFT) [44]. By calculating the electronic structure of our lead compounds with the ORCA program [42], we could determine their Frontier Molecular Orbitals (HOMO and LUMO). The energy gap between these orbitals is a powerful indicator of a molecule's chemical reactivity [43, 65].

3. RESULTS

Our investigation began by identifying a diverse chemical arsenal within Piper chaba. From this library, a select group of 15 compounds, including the prominent alkaloid Piperine, stood out during our initial screening. When subjected to a virtual ADME trial, these molecules demonstrated highly favorable, drug-like characteristics. Most notably, they passed key checkpoints like Lipinski's rule of five, signaling a strong potential for oral bioavailability. For any aspiring anti-Alzheimer's drug, the ability to cross the blood-brain barrier (BBB) is the first and most critical hurdle. Our analysis predicted that several of the lead compounds, including piperine, could indeed make this crucial journey into the central nervous system. Combined with a low predicted toxicity profile, these foundational properties gave us the green light to advance these promising candidates. The key pharmacokinetic data are summarized in Table 1.

Table 1: Predicted ADME and Drug-Likeness Properties of Key Piper chaba Phytochemicals

Compound	Formula	MW (g/mol)	H-Bond Donors	H-Bond Acceptors	TPSA (Å²)	LogP	BBB Permeant	Lipinski Violations
Piperine	C17H19NO3	285.34	0	3	45.4	2.5	Yes	0
Methylpiperate	C15H17NO4	287.30	1	4	63.3	2.1	Yes	0
Piperlongumine	C17H19NO5	317.34	1	5	72.8	1.8	Yes	0
Bicyclo[7.2.0]undec-4-ene	C15H24	204.35	0	0	0.0	4.6	Yes	1 (LogP> 4.5)
(E)-3-Butylidene-4,5-dihydr	C12H14O2	190.24	0	2	26.3	2.47	Yes	0

oisobenzofuran-1(3H)-one								

Our network analysis acted as a treasure map, guiding us from a broad list of hundreds of potential protein targets to the most critical players in the disease. After cross-referencing with AD-specific gene databases, we pinpointed a network of 60 relevant proteins. Within this network, a clear hierarchy emerged. By analyzing the connections between these proteins, we identified three "hub proteins" that stood out as the most influential command-and-control centers: PTGS2 (COX-2), a master regulator of inflammation; PLA2G4A, a key enzyme that acts just upstream of PTGS2; and CYP2C19, a vital metabolic enzyme.

Zooming in from the network map to the atomic landscape, our molecular docking simulations provided the first visual confirmation of our hypothesis. Lead compounds from P. chaba, especially piperine and Bicyclo[7.2.0]undec-4-ene, fit beautifully into the active sites of our three hub proteins. They achieved binding energy scores that were not only strong but often superior to those of known reference drugs, like aspirin for PTGS2 (Table 2). A closer look at the docking poses revealed the specific interactions responsible for this tight binding, which are detailed in Table 3.

Table 2: Molecular Docking Scores (kcal/mol) of Lead Phytochemicals with Hub Proteins

Ligand	**PTGS2 (PDB: 5F19)**	**PLA2G4A (PDB: 1CJY)**	**CYP2C19 (PDB: 4GQS)**
Piperine	**-9.2**	**-8.8**	**-8.5**
Bicyclo[7.2.0]undec-4-ene	**-8.0**	**-7.5**	**-7.2**
Methylpiperate	-8.5	-8.1	-7.9
(E)-3-Butylidene...one	-7.8	-7.2	-6.9
Aspirin (Control)	*-6.4*	*N/A*	*N/A*

Table 3: Detailed Interaction Analysis of Top Protein-Ligand Complexes from Molecular Docking

Protein	**Ligand**	**Binding Energy (kcal/mol)**	**Key Interacting Residues**	**Type of Interaction**
PTGS2	**Piperine**	**-9.2**	TRP387, PHE518, VAL349, LEU352	Pi-Alkyl, Alkyl
			SER530, TYR385	Hydrogen Bond (conventional)
	Bicyclo[7.2.0]...	-8.0	TRP387, PHE518	Pi-Alkyl

			VAL523, LEU352, ALA527	Alkyl
PLA2G4A	**Piperine**	**-8.8**	HIS257, ASP549	Hydrogen Bond
			LEU136, VAL114, PRO81	Alkyl, Pi-Alkyl
CYP2C19	**Piperine**	**-8.5**	PHE114, LEU366, ALA297	Pi-Alkyl
			ASN107	Carbon-Hydrogen Bond

The static image from docking was compelling, but the moving picture from our 100 ns MD simulations was definitive. We watched as the top-performing complexes, Piperine-PTGS2 and Piperine-PLA2G4A, held together with remarkable stability over the entire simulation. The RMSD plots showed that the structures of the complexes remained rock-solid, fluctuating only minimally. We also observed that the amino acids in the binding pocket became less "wiggly" when piperine was present, a "clamping down" effect that further confirmed a persistent and stable binding event. Our final MM/PBSA calculations produced highly negative binding free energies (-158.3 kJ/mol for Piperine-PTGS2), confirming that the formation of these complexes is a highly spontaneous and energetically favorable process.

Our final analysis took us into the quantum world. DFT calculations provided insight into the electronic nature of our lead compounds. The results, summarized in Table 4, show that piperine possesses a HOMO-LUMO energy gap in the "sweet spot" for biological activity—indicative of a molecule that is reactive enough to engage its target but stable enough to be a viable drug.

Table 4: DFT Calculation Results for Lead Compounds

Compound	HOMO (eV)	LUMO (eV)	Energy Gap (eV)	Hardness (η)	Softness (S)
Piperine	**-5.89**	**-1.79**	**4.10**	**2.05**	**0.488**
Bicyclo[7.2.0]...	-6.15	0.27	6.42	3.21	0.311
(E)-3-Butylidene...	-6.09	-2.05	4.04	2.02	0.495

DISCUSSION

The story of Alzheimer's drug discovery has been a humbling lesson in the dangers of oversimplification. The failure of countless single-target drugs has taught us that we cannot fight a multi-front war with a single weapon [7, 47]. Our study was designed with this lesson in mind. By using an integrated computational approach, we sought to understand how Piper chaba might work not as a single "magic bullet," but as a sophisticated, multi-compound formulation capable of modulating a whole network of disease processes. Our findings strongly endorse this view, revealing P. chaba phytochemicals as potent agents that can simultaneously target several key drivers of AD.

The journey from a plant on a vine to a pill in a bottle is long, and it begins with fundamental questions of drug-likeness. Our ADME screening was the first critical gate, and the results were exceptionally positive. The finding that piperine and other lead compounds are predicted to be orally bioavailable and, crucially, able to cross the blood-brain barrier is of immense practical importance [28]. The BBB is the brain's fortress wall, and the ability to breach it is a non-negotiable requirement for any anti-AD drug. This favorable pharmacokinetic profile provides the solid foundation on which the plausibility of our entire

hypothesis rests.

Our network pharmacology analysis acted as an unbiased guide, illuminating the most critical nodes in the disease network. The emergence of PTGS2 (COX-2) and PLA2G4A as the top two hubs was a powerful finding. It confirms that the anti-inflammatory properties of P. chaba are not just a peripheral effect but are likely central to its therapeutic potential. Neuroinflammation is no longer seen as a mere bystander in AD; it is a core driver of the pathology [2, 46]. PTGS2 (COX-2) is the engine of the inflammatory response in the AD brain, churning out toxic molecules that damage neurons [51, 52, 53]. Our simulations, which showed piperine docking tightly and stably into the PTGS2 active site, provide a clear, atom-level picture of how this engine could be switched off. PLA2G4A is the enzyme that provides the fuel for that fire [50]. It also plays a sinister secondary role by damaging the cell's waste-disposal machinery (autophagy), leading to a buildup of toxic garbage [54]. The ability of piperine to also bind strongly to PLA2G4A suggests a brilliant two-for-one mechanism: it can cut the fuel line to the inflammatory engine while also helping to get the cellular housekeeping services back online.

The identification of the metabolic enzyme CYP2C19 as a third hub added another fascinating layer to our story. While best known for their role in the liver, these enzymes are also active in the brain, influencing everything from the levels of neuroprotective hormones to the metabolism of fats that are critical for brain health [55]. With recent genetic studies linking CYP2C19 variants directly to AD risk [49], our finding that P. chaba compounds interact with this enzyme suggests a third, metabolic dimension to their therapeutic potential.

A key strength of our study is that we didn't stop at prediction. We rigorously tested our hypotheses at multiple scales. The high-affinity docking scores gave us the first hint of a strong interaction [56]. But it was the 100 ns MD simulations that provided the "smoking gun" [61, 62]. Watching the piperine-protein complexes remain steadfast and stable over this extended timescale transforms the finding from a computational prediction into a physically plausible and dynamically robust event [57, 66]. This is the kind of strong, atom-level evidence needed to build a compelling case for a new drug mechanism.

It is crucial to approach these exciting results with scientific humility. A computational study, no matter how sophisticated, is a map, not the territory itself. The hypotheses generated here, while strong, now need to be tested in the real world of the laboratory. The path forward is clear: in vitro biochemical assays, followed by cell-based models, and finally, testing in transgenic animal models of AD. This structured pipeline is the essential next chapter in translating the promise of Piper chaba into a potential therapy for those suffering from Alzheimer's disease.

5. CONCLUSION

In this study, we journeyed from the traditional knowledge of a medicinal plant to the cutting edge of computational drug discovery. Our comprehensive in silico investigation has built a powerful, multi-layered case for the therapeutic potential of Piper chaba Hunter in the fight against Alzheimer's disease. We have shown that this plant contains a sophisticated arsenal of bioactive compounds, led by piperine, that can wage a multi-pronged assault on the disease. By simultaneously targeting the critical hubs of neuroinflammation (PTGS2, PLA2G4A) and metabolic dysregulation (CYP2C19), these phytochemicals have the potential to disrupt the vicious cycles that drive neurodegeneration.

Our work provides more than just a list of promising compounds; it offers a clear, mechanistically plausible, and atomistically detailed blueprint for how they might work. This study provides a rational foundation for prioritizing Piper chaba and its constituents for further laboratory investigation and preclinical development. It is a testament to the immense therapeutic potential that still lies hidden in nature, and a powerful example of how modern computational science can help us to unlock it.

6. REFERENCES

[1] Z. Breijyeh and R. Karaman, “Comprehensive Review on Alzheimer’s Disease: Causes and Treatment,” Molecules 25, no. 24 (2020): 5789, https://doi.org/10.3390/molecules25245789.

[2] M. U. Ali, L. Anwar, M. H. Ali, et al., “Signalling Pathways Involved in Microglial Activation in Alzheimer’s Disease and Potential Neuroprotective Role of Phytoconstituents,” CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders) 23, no. 7 (2024): 819–840, https://doi.org/10.2174/1871527322666221223091529.

[3] N. Bogdanovic, O. Hansson, H. Zetterberg, et al., “Alzheimer's Disease-The Most Common Cause of Dementia,” Lakartidningen 117 (2020).

[4] M. A. Rahman, M. H. Rahman, A. Mamun-Or-Rashid, et al., “Autophagy Modulation in Aggresome Formation: Emerging Implications and Treatments of Alzheimer’s Disease,” Biomedicines 10, no. 5 (2022): 1027, https://doi.org/10.3390/biomedicines10051027.

[5] J. Weller and A. Budson, “Current Understanding of Alzheimer’s Disease Diagnosis and Treatment,” F1000Research 7 (2018): 1161, https://doi.org/10.12688/f1000research.14506.1.

[6] B. T. Winslow, M. K. Onysko, C. M. Stob, and K. A. Hazlewood, “Treatment of Alzheimer Disease,” Am Fam Physician 83, no. 12 (2011): 1403–1412.

[7] K. G. Yiannopoulou, A. I. Anastasiou, V. Zachariou, and S. H. Pelidou, “Reasons for Failed Trials of Disease-Modifying Treatments for Alzheimer Disease and Their

Contribution in Recent Research," Biomedicines 7, no. 4 (2019): 97, https://doi.org/10.3390/biomedicines7040097.

[8] K. R. Ahmed, M. M. Rahman, M. N. Islam, M. M. H. Fahim, M. A. Rahman, and B. Kim, "Antioxidants Activities of Phytochemicals Perspective Modulation of Autophagy and Apoptosis to Treating Cancer," Biomedicine & Pharmacotherapy 174 (2024): 116497, https://doi.org/10.1016/j.biopha.2024.116497.

[9] M. J. R. Howes, N. S. Perry, and P. J. Houghton, "Plants With Traditional Uses and Activities, Relevant to the Management of Alzheimer's Disease and Other Cognitive Disorders," Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 17, no. 1 (2003): 1–18, https://doi.org/10.1002/ptr.1280.

[10] D. O. Kennedy and E. L. Wightman, "Herbal Extracts and Phytochemicals: Plant Secondary Metabolites and the Enhancement of Human Brain Function," Advances in Nutrition 2, no. 1 (2011): 32–50, https://doi.org/10.3945/an.110.000117.

[11] V. Kumar, "Potential Medicinal Plants for CNS Disorders: An Overview," Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 20, no. 12 (2006): 1023–1035, https://doi.org/10.1002/ptr.1970.

[12] B. J. Kelley and D. S. Knopman, "Alternative Medicine and Alzheimer Disease," Neurologist 14, no. 5 (2008): 299–306, https://doi.org/10.1097/NRL.0b013e318172cf4d.

[13] A. K. Singhal, V. Naithani, and O. P. Bangar, "Medicinal Plants With a Potential to Treat Alzheimer and Associated Symptoms," International Journal of Nutrition, Pharmacology, Neurological Diseases 2, no. 2 (2012): 84–91, https://doi.org/10.4103/2231-0738.95927.

[14] I. Zeouk, W. Ouedrhiri, C. Rais, C. Slimani, and K. Bekhti, "Phytochemical Quantification and Optimization of Synergistic Antioxidant Potency of Moroccan Medicinal Plants Using Constrained Mixture Design," Advances in Traditional Medicine 24, no. 4 (2024): 1037–1043, https://doi.org/10.1007/s13596-024-00754-1.

[15] R. V. Rao, O. Descamps, V. John, and D. E. Bredesen, "Ayurvedic Medicinal Plants for Alzheimer's Disease: A Review," Alzheimer's Research & Therapy 4 (2012): 1–9.

[16] M. Taufiq-Ur-Rahman, J. A. Shilpi, M. Ahmed, and C. F. Hossain, "Preliminary Pharmacological Studies on Piper Chaba Stem Bark," Journal of Ethnopharmacology 99, no. 2 (2005): 203–209.

[17] Z. Jin, G. Borjihan, R. Zhao, Z. Sun, G. B. Hammond, and T. Uryu, "Antihyperlipidemic Compounds From the Fruit of Piper longum L," Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 23, no. 8 (2009): 1194–1196, https://doi.org/10.1002/ptr.2630.

[18] S. A. Lee, J. S. Hwang, X. H. Han, et al., "Methylpiperate Derivatives From Piper longum and Their Inhibition of Monoamine Oxidase," Archives of Pharmacal Research 31, no. 6 (2008): 679–683, https://doi.org/10.1007/s12272-001-1212-7.

[19] S. Sireeratawong, A. Itharat, N. Lerdvuthisopon, et al., "Anti-Inflammatory, Analgesic, and Antipyretic Activities of the Ethanol Extract of Piper interruptum Opiz. and Piper chaba Linn," International Scholarly Research Notices 2012, 6 https://doi.org/10.5402/2012/480265.

[20] L. Shen, W. Chen, B. Zhang, L. Liu, and Y. Cao, "Integrating Network Pharmacology and Bioinformatics Analysis to Explore the Mechanism of Yupingfengsan in Treating Lung Adenocarcinoma," European Journal of Integrative Medicine 31 (2019): 100967, https://doi.org/10.1016/j.eujim.2019.100967.

[21] W. Guo, J. Huang, N. Wang, et al., "Integrating Network Pharmacology and Pharmacological Evaluation for Deciphering the Action Mechanism of Herbal Formula Zuojin Pill in Suppressing Hepatocellular Carcinoma," Frontiers in Pharmacology 10 (2019): 1185, https://doi.org/10.3389/fphar.2019.01185.

[22] A. L. Hopkins, "Network Pharmacology," Nature Biotechnology 25, no. 10 (2007): 1110–1111.

[23] B. Patwardhan and R. Chaguturu, Innovative Approaches in Drug Discovery: Ethnopharmacology, Systems Biology and Holistic Targeting (Elsevier, 2016).

[24] B. Zhang, X. Wang, and S. Li, "An Integrative Platform of TCM Network Pharmacology and its Application on a Herbal Formula, Qing-Luo-Yin," Evidence-Based Complementary and Alternative Medicine 2013 (2013): 12, 456747, https://doi.org/10.1155/2013/456747.

[25] S. I. Berger and R. Iyengar, "Network Analyses in Systems Pharmacology," Bioinformatics 25, no. 19 (2009): 2466–2472, https://doi.org/10.1093/bioinformatics/btp465.

[26] R. Islam, M. S. Rahman, and S. M. Rahman, "GC-MS Analysis and Antibacterial Activity of Cuscuta reflexa Against Bacterial Pathogens," Asian Pacific Journal of Tropical Disease 5, no. 5 (2015): 399–403, https://doi.org/10.1016/S2222-1808(14)60804-5.

[27] M. N. H. Zilani, M. A. Islam, P. Biswas, et al., "Metabolite Profiling, Anti-Inflammatory, Analgesic Potentials of Edible Herb Colocasia gigantea and Molecular Docking Study Against COX-II Enzyme," Journal of Ethnopharmacology 281 (2021): 114577, https://doi.org/10.1016/j.jep.2021.114577.

[28] A. Daina, O. Michielin, and V. Zoete, "SwissADME: A Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small

Molecules," Scientific Reports 7, no. 1 (2017): 42717, https://doi.org/10.1038/srep42717.

[29] S. Kim, J. Chen, T. Cheng, et al., "PubChem 2023 Update," Nucleic Acids Research 51, no. D1 (2023): D1373–D1380, https://doi.org/10.1093/nar/gkac956.

[30] M. J. Keiser, B. L. Roth, B. N. Armbruster, P. Ernsberger, J. J. Irwin, and B. K. Shoichet, "Relating Protein Pharmacology by Ligand Chemistry," Nature Biotechnology 25, no. 2 (2007): 197–206.

[31] A. Daina, O. Michielin, and V. Zoete, "SwissTargetPrediction: Updated Data and New Features for Efficient Prediction of Protein Targets of Small Molecules," Nucleic Acids Research 47, no. W1 (2019): W357–W364, https://doi.org/10.1093/nar/gkz382.

[32] A. Hamosh, A. F. Scott, J. S. Amberger, C. A. Bocchini, and V. A. McKusick, "Online Mendelian Inheritance in Man (OMIM), a Knowledgebase of Human Genes and Genetic Disorders," Nucleic Acids Research 33 (2005): D514–D517.

[33] J. Piñero, J. M. Ramírez-Anguita, J. Saüch-Pitarch, et al., "The DisGeNET Knowledge Platform for Disease Genomics: 2019 Update," Nucleic Acids Research 48 (2020): D845–D855.

[34] K. Blennow, M. J. de Leon, and H. Zetterberg, "Alzheimer's Disease," Lancet 368, no. 9533 (2006): 387–403, https://doi.org/10.1016/S0140-6736(06)69113-7.

[35] S. Dallakyan and A. J. Olson, "Small-Molecule Library Screening by Docking With PyRx," Methods Mol Biol 1263 (2015): 243–250, https://doi.org/10.1007/978-1-4939-2269-7_19.

[36] H. Land and M. S. Humble, "YASARA: A Tool to Obtain Structural Guidance in Biocatalytic Investigations," in Protein Engineering: Methods and Protocols (Springer New York, 2017), 43–67.

[37] J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman, and D. A. Case, "Development and Testing of a General Amber Force Field," Journal of Computational Chemistry 25, no. 9 (2004): 1157–1174.

[38] M. F. Harrach and B. Drossel, "Structure and Dynamics of TIP3P, TIP4P, and TIP5P Water Near Smooth and Atomistic Walls of Different Hydroaffinity," Journal of Chemical Physics 140, no. 17 (2014): 174501, https://doi.org/10.1063/1.4872239.

[39] H. J. Berendsen, W. F. van Gunsteren, A. DiNola, and J. R. Haak, "Molecular Dynamics With Coupling to an External Bath," Journal of Chemical Physics 81, no. 8 (1984): 3684–3690, https://doi.org/10.1063/1.448118.

[40] M. J. Harvey and G. De Fabritiis, "An Implementation of the Smooth Particle Mesh Ewald Method on GPU Hardware," Journal of Chemical Theory and Computation 5, no. 9 (2009): 2371–2377, https://doi.org/10.1021/ct900275y.

[41] M. D. Hanwell, D. E. Curtis, D. C. Lonie, T. Vandermeersch, E. Zurek, and G. R. Hutchison, "Avogadro: An Advanced Semantic Chemical Editor, Visualization, and Analysis Platform," Journal of Cheminformatics 4 (2012): 1–17.

[42] F. Neese, "Software Update: The ORCA Program System—-Version 5.0," Wiley Interdisciplinary Reviews: Computational Molecular Science 12 (2022): e1606.

[43] R. G. Pearson, "Absolute Electronegativity and Hardness Correlated With Molecular Orbital Theory," Proceedings of the National Academy of Sciences 83, no. 22 (1986): 8440–8441, https://doi.org/10.1073/pnas.83.22.8440.

[44] R. G. Parr, "Density Functional Theory of Atoms and Molecules," in Horizons of Quantum Chemistry, eds. K. Fukui and B. Pullman (Springer, 1980), 5–15.

[45] K.-K. Oh, M. Adnan, and D.-H. Cho, "Network Pharmacology Study to Interpret Signaling Pathways of Ilex cornuta Leaves Against Obesity," Processes 9, no. 7 (2021): 1106, https://doi.org/10.3390/pr9071106.

[46] P. Talwar, J. Sinha, S. Grover, et al., "Dissecting Complex and Multifactorial Nature of Alzheimer's Disease Pathogenesis: A Clinical, Genomic, and Systems Biology Perspective," Molecular Neurobiology 53, no. 7 (2016): 4833–4864, https://doi.org/10.1007/s12035-015-9390-0.

[47] C. Nogales, Z. M. Mamdouh, M. List, C. Kiel, A. I. Casas, and H. H. H. W. Schmidt, "Network Pharmacology: Curing Causal Mechanisms Instead of Treating Symptoms," Trends in Pharmacological Sciences 43, no. 2 (2022): 136–150, https://doi.org/10.1016/j.tips.2021.11.004.

[48] Y. Dong, P. Qiu, R. Zhu, et al., "A Combined Phytochemistry and Network Pharmacology Approach to Reveal the Potential Antitumor Effective Substances and Mechanism of Phellinus igniarius," Frontiers in Pharmacology 10 (2019): https://doi.org/10.3389/fphar.2019.00266.

[49] A. L. Benedet, L. Yu, A. Labbe, et al., "CYP2C19 Variant Mitigates Alzheimer Disease Pathophysiology in vivo and Post-mortem," Neurology: Genetics 4, no. 1 (2018): e216, https://doi.org/10.1212/NXG.0000000000000216.

[50] J. C. Lee, A. Simonyi, A. Y. Sun, and G. Y. Sun, "Phospholipases A2 and Neural Membrane Dynamics: Implications for Alzheimer's Disease," J Neurochem 116, no. 5 (2011): 813–819, https://doi.org/10.1111/j.1471-4159.2010.07033.x.

[51] S. L. Ma, N. L. Tang, Y. P. Zhang, et al., "Association of Prostaglandin-Endoperoxide Synthase 2 (PTGS2) Polymorphisms and Alzheimer's Disease in Chinese," Neurobiol Aging 29, no. 6 (2008): 856–860.

[52] A. Greenhough, H. J. Smartt, A. E. Moore, et al., "The COX-2/PGE 2 Pathway: Key Roles in the Hallmarks of Cancer and Adaptation to the Tumour Microenvironment,"

Carcinogenesis 30, no. 3 (2009): 377–386, https://doi.org/10.1093/carcin/bgp014.

[53] D. T. Stark and N. G. Bazan, "Synaptic and Extrasynaptic NMDA Receptors Differentially Modulate Neuronal Cyclooxygenase-2 Function, Lipid Peroxidation, and Neuroprotection," Journal of Neuroscience 31, no. 39 (2011): 13710–13721, https://doi.org/10.1523/JNEUROSCI.3544-11.2011.

[54] C. Sarkar, J. W. Jones, N. Hegdekar, et al., "PLA2G4A/cPLA2-Mediated Lysosomal Membrane Damage Leads to Inhibition of Autophagy and Neurodegeneration After Brain Trauma," Autophagy 16, no. 3 (2020): 466–485, https://doi.org/10.1080/15548627.2019.1628538.

[55] S. Miksys and R. F. Tyndale, "Cytochrome P450–mediated drug metabolism in the brain," Journal of Psychiatry and Neuroscience 38, no. 3 (2013): 152–163, https://doi.org/10.1503/jpn.120133.

[56] X. Du, Y. Li, Y. L. Xia, et al., "Insights Into Protein–Ligand Interactions: Mechanisms, Models, and Methods," International Journal of Molecular Sciences 17, no. 2 (2016): 144, https://doi.org/10.3390/ijms17020144.

[57] A. J. Owoloye, F. C. Ligali, O. A. Enejoh, et al., "Molecular Docking, Simulation and Binding Free Energy Analysis of Small Molecules as PfHT1 Inhibitors," PLoS One 17, no. 8 (2022): e0268269, https://doi.org/10.1371/journal.pone.0268269.

[58] M. Hasan, M. Tariquzzaman, M. R. Islam, T. F. Susmi, M. S. Rahman, and M. S. Rahi, "Plant-Derived Bisphenol C Is a Drug Candidate Against Nipah Henipavirus Infection: An In-Vitro and In-Silico Study of Pouzolzia zeylanica (L.) Benn," In Silico Pharmacology 13, no. 1 (2025): 43, https://doi.org/10.1007/s40203-025-00328-2.

[59] M. Aamir, V. K. Singh, M. K. Dubey, et al., "In Silico Prediction, Characterization, Molecular Docking, and Dynamic Studies on Fungal SDRs as Novel Targets for Searching Potential Fungicides Against Fusarium Wilt in Tomato," Frontiers in Pharmacology 9 (2018): 1038, https://doi.org/10.3389/fphar.2018.01038.

[60] P. Sonkar, S. Purwar, P. Bhargva, et al., "In Silico Profiling, Docking Analysis, and Protein Interactions of Secondary Metabolites in Musa spp. Against the SGE1 Protein of Fusarium Oxysporum f. sp. cubense," Computational Biology and Chemistry 113 (2024): 108230, https://doi.org/10.1016/j.compbiolchem.2024.108230.

[61] S. AlRawashdeh and K. H. Barakat, "Applications of Molecular Dynamics Simulations in Drug Discovery," Methods Mol Biol 2714 (2024): 127–141, https://doi.org/10.1007/978-1-0716-3441-7_7.

[62] J. D. Durrant and J. A. McCammon, "Molecular Dynamics Simulations and Drug Discovery," BMC Biology 9, no. 1 (2011): 71, https://doi.org/10.1186/1741-7007-9-71.

[63] M. N. Arthur, K. Bebla, E. Broni, et al., "Design of Inhibitors That Target the Menin-Mixed-Lineage Leukemia Interaction," Computation 12, no. 1 (2024): 3, https://doi.org/10.3390/computation12010003.

[64] D. R. Houston and M. D. Walkinshaw, "Consensus Docking: Improving the Reliability of Docking in a Virtual Screening Context," Journal of Chemical Information and Modeling 53, no. 2 (2013): 384–390, https://doi.org/10.1021/ci300399w.

[65] M. Miar, A. Shiroudi, K. Pourshamsian, A. R. Oliaey, and F. Hatamjafari, "Theoretical Investigations on the HOMO–LUMO Gap and Global Reactivity Descriptor Studies, Natural Bond Orbital, and Nucleus-Independent Chemical Shifts Analyses of 3-Phenylbenzo[d]Thiazole-2(3H)-Imine and its Para-Substituted Derivatives: Solvent and Substituent Effects," Journal of Chemical Research 45 (2020): 147–158.

[66] S. Akash, F. I. Aovi, M. A. K. Azad, et al., "A Drug Design Strategy Based on Molecular Docking and Molecular Dynamics Simulations Applied to Development of Inhibitor Against Triple-Negative Breast Cancer by Scutellarein Derivatives," PLoS One 18, no. 10 (2023): e0283271, https://doi.org/10.1371/journal.pone.0283271.

[67] C. N. Cavasotto, M. G. Aucar, and N. S. Adler, "Computational Chemistry in Drug Lead Discovery and Design," International Journal of Quantum Chemistry 119, no. 2 (2019): e25678, https://doi.org/10.1002/qua.25678.

[68] L. S. Hashmi, M. A. Hossain, A. M. Weli, Q. Al-Riyami, and J. N. Al-Sabahi, "Gas Chromatography-Mass Spectrometry Analysis of Different Organic Crude Extracts From the Local Medicinal Plant of Thymus vulgaris L," Asian Pacific Journal of Tropical Biomedicine 3, no. 1 (2013): 69–73, https://doi.org/10.1016/S2221-1691(13)60026-X.

[69] M. M. Rahman, S. H. Ahmad, M. T. Mohamed, and M. Z. Ab Rahman, "Antimicrobial Compounds From Leaf Extracts of Jatropha curcas, Psidium guajava, and Andrographis paniculata," Scientific World Journal 2014 (2014): 635240, https://doi.org/10.1155/2014/635240.

[70] A. Thomas, H. D. Mazigo, A. Manjurano, D. Morona, and E. J. Kweka, "Evaluation of Active Ingredients and Larvicidal Activity of Clove and Cinnamon Essential Oils Against Anopheles gambiae (Sensu Lato)," Parasit Vectors 10, no. 1 (2017): 411, https://doi.org/10.1186/s13071-017-2355-6.

[71] M. Soheili, M. Karimian, G. Hamidi, and M. Salami, "Alzheimer's Disease Treatment: The Share of Herbal Medicines," Iranian Journal of Basic Medical Sciences 24 (2021): 123.

[72] S. Shoaib, M. A. Ansari, A. A. Fatease, et al., "Plant-Derived Bioactive Compounds in the Management of Neurodegenerative Disorders: Challenges, Future

Directions and Molecular Mechanisms Involved in Neuroprotection," Pharmaceutics 15, no. 3 (2023): 749, https://doi.org/10.3390/pharmaceutics15030749.

[73] T. Tuzimski and A. Petruczynik, "Determination of Anti-Alzheimer's Disease Activity of Selected Plant Ingredients," Molecules 27, no. 10 (2022): 3222, https://doi.org/10.3390/molecules27103222.