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INSILICO IDENTIFICATION OF THE ANTI-ALZHEIMER POTENTIAL OF *OCIMUM SANCTUM* PHYTOCOMPOUND VIA DOCKING, ADMET PROFILING AND NETWORK PHARMACOLOGY

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ABSTRACT
Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid-β aggregation, tau hyperphosphorylation, oxidative stress, and chronic neuroinflammation. Current pharmacotherapies provide only symptomatic relief and are often associated with adverse effects, necessitating safer multi-target therapeutic strategies. This study aimed to investigate the anti-Alzheimer potential of *Ocimum sanctum* phytocompounds using an integrated in-silico approach involving molecular docking, ADMET profiling, toxicity prediction, and network pharmacology. Fourteen ligands, including ten phytochemicals and four standard drugs, were evaluated using SwissADME, pkCSM, and StopTox. Network pharmacology identified 64 overlapping targets between phytocompounds and AD-associated genes, with SRC, PTGS2, ESR1, MAPK3, and GSK3B emerging as key hub proteins. Molecular docking analysis revealed strong binding affinities of several phytoconstituents toward these targets. Ursolic acid demonstrated the highest multi-target potential, particularly against PTGS2 (−10.8 kcal/mol) and SRC (−9.8 kcal/mol), outperforming reference drugs in certain interactions. ADMET predictions indicated favorable pharmacokinetic properties for most compounds, with acceptable absorption and blood–brain barrier permeability, while toxicity profiling suggested a generally safe therapeutic window for selected candidates. Collectively, the findings highlight *O. sanctum* phytochemicals, especially ursolic acid, as promising multi-target modulators against AD pathology, warranting further in vitro and in vivo validation.

KEYWORDS: Alzheimer's disease; Ocimum sanctum; phytochemicals; molecular docking; network pharmacology; ADMET analysis; SwissADME; protein–protein interaction

INTRODUCTION
Alzheimer's disease (AD) is the most common cause of dementia and represents one of the greatest global public health challenges due to increasing life expectancy and population aging. It is a progressive neurodegenerative disorder characterized by gradual deterioration of memory, cognitive function, language, executive abilities, and behavioral performance, ultimately resulting in complete dependence and reduced quality of life (Knopman et al., 2021). According to recent epidemiological reports, more than 57 million individuals worldwide are living with dementia, with Alzheimer's disease accounting for approximately 60–70% of all cases (Lanctot et al., 2024). The prevalence of AD is expected to increase dramatically over the coming decades, creating substantial socioeconomic and healthcare burdens, particularly in low- and middle-income countries where diagnostic and therapeutic resources remain limited. In Pakistan, dementia affects approximately 150,000–200,000 individuals, highlighting the urgent need to develop affordable and effective therapeutic strategies for neurodegenerative disorders (Ali et al., 2023).

The pathological progression of Alzheimer's disease is highly complex and involves numerous interconnected molecular mechanisms rather than a single causative pathway (Issa, 2025). The disease is primarily characterized by extracellular deposition of amyloid-β (Aβ) plaques, intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein, progressive synaptic dysfunction, neuronal degeneration, mitochondrial impairment, oxidative stress, chronic neuroinflammation, calcium dysregulation, and cholinergic neurotransmission deficits (Zheng & Wang, 2025). (Kumar et al., 2024). In addition to these pathological hallmarks, increasing evidence suggests that dysregulation of multiple intracellular signaling pathways including mitogen-activated protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K)/Akt, glycogen synthase kinase-

3β (GSK3β), estrogen receptor signaling (ESR1), and Src-family kinases (SRC) plays a significant role in disease initiation and progression (Hampel et al., 2018).
Consequently, Alzheimer's disease is increasingly recognized as a multifactorial disorder requiring therapeutic agents capable of simultaneously modulating multiple biological targets.
Current pharmacological management of Alzheimer's disease primarily consists of acetylcholinesterase inhibitors (donepezil, rivastigmine, and galantamine) and the N-methyl-D-aspartate (NMDA) receptor antagonist memantine (Pathak & Kabra, 2024). Although these medications temporarily improve cognitive symptoms and daily functioning, they neither halt nor reverse neurodegeneration (Rull et al., 2021). Furthermore, long-term administration is frequently associated with adverse gastrointestinal, cardiovascular, and neurological effects, resulting in poor patient compliance (Yang et al., 2024). Recently approved anti-amyloid monoclonal antibodies have demonstrated modest clinical benefits. However, their high cost, limited accessibility, potential adverse effects, and strict patient eligibility criteria restrict widespread clinical application (Lusk et al., 2025). These limitations emphasize the continued need for safer, affordable, and multi-target therapeutic candidates capable of interfering with several pathological mechanisms simultaneously (Hossain et al., 2025). Natural compounds with multi-target potential have attracted attention as alternative therapeutic strategies.
Medicinal plants remain a rich source of bioactive compounds for drug discovery, and Ocimum sanctum (Holy Basil/Tulsi) is especially notable for its antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, and neuroprotective properties in traditional Ayurvedic medicine (Parween et al., 2025). Phytochemical studies have identified constituents such as ursolic acid, eugenol, apigenin, luteolin, orientin, vitexin, rosmarinic acid, caffeic acid, linalool, and carvacrol, several of which individually show antioxidant activity, suppression of neuroinflammatory mediators, reduced amyloid aggregation, and protection against neuronal apoptosis (Vasincu et al., 2025), positioning O. sanctum as a candidate source of multi-target Alzheimer's disease (AD) therapeutics.
Computational pharmacology particularly network pharmacology combined with molecular docking and ADMET prediction has accelerated early drug discovery by revealing how natural compounds act on multiple targets and pathways simultaneously, rather than the conventional "one drug–one target" model (Islam et al., 2024). Prior studies applying tools such as Swiss Target Prediction, STRING, and Cyto-scape to other medicinal plants against AD (e.g., Cymbopogon citratus, Convolvulus pluricaulis, cinnamon species) have successfully identified hub genes and validated ligand target interactions through docking (Chen et al., 2024). However, most existing work on O. sanctum phytochemicals has examined single compounds or single targets in isolation, without an integrated workflow combining target prediction, protein-protein interaction analysis, hub gene screening, pharmacokinetics, and toxicity profiling.
To address this gap, the present study applies an integrated in silico strategy Swiss Target Prediction, Gene Cards, STRING, Cytoscape/CytoHubba, molecular docking, Swiss ADME, pkCSM, and StopTox to evaluate ten major O. sanctum phytochemicals alongside four approved anti-AD drugs, focusing on five AD-relevant targets (SRC, PTGS2, ESR1, MAPK3, GSK3β). Rather than proving efficacy, the goal is to generate mechanistic hypotheses and identify promising multi-target leads for future experimental validation.

MATERIALS AND METHODS

Acquiring the Selected Phytochemicals' Structural Data and SMILES Codes

The study focuses on phytochemicals from *O. sanctum*, which were chosen based on prior research demonstrating their medicinal potential against Alzheimer's disease. The PubChem database (National Center for Biotechnology Information, 2023) https://pubchem.ncbi.nlm.nih.gov/ supplied SMILES notations for these substances, which were used to assess their physicochemical and pharmacokinetic features, such as lipophilicity, drug-likeness, water solubility, and predicted toxicity. Table 1 provides detailed information on the compounds, such as chemical formulas, molecular structures, and SMILES strings.

Assessment of the Chemical Properties and Pharmacokinetic Profiles of Phytochemical Agents

The physicochemical parameters, including molecular weight, lipophilicity (LogP), aqueous solubility, and drug-likeness, along with pharmacokinetic characteristics such as absorption, distribution, metabolism, excretion, and toxicity (ADMET), were evaluated using web-based computational tools. SwissADME (http://www.swissadme.ch/) (Daina et al., 2017) and pkCSM (https://biosig.lab.uq.edu.au/pkcsm/) (Pires et al., 2015) platforms were employed to predict these properties for the phytoconstituents of *O. sanctum* (Tulsi) as shown in Table 2-6 in order to assess their potential as anti-Alzheimer’s agents.

Analysis of the Drug-Likeness Potential of the Phytocompounds

Six predetermined sets of criteria were used to assess the chosen compounds' drug-likeness. These included Veber's rule, Egan's rule, Muegge's rule, Ghose's filter, and Lipinski's Rule of Five. These recommendations are all predicated on particular physicochemical characteristics that aid in identifying whether a compound has qualities appropriate for medication development and discovery. The SwissADME web tool (http://www.swissadme.ch/) was used to evaluate the phytocompounds' drug-likeness.

Evaluation of the Toxicological Profile of Phytocompounds

Toxicity prediction of the phytochemical compounds derived from *O. sanctum* was Performed using independent web-based tool, sTopTox (https://stoptox.mml.unc.edu/).
sTopTox utilizes quantitative structure–activity relationship (QSAR) models for in silico toxicity screening (Borba et al., 2022). The combined approach enhanced the accuracy of toxicity profiling of *O. sanctum*

phytocompounds, ensuring the selection of candidates with favorable safety margins for potential therapeutic application against Alzheimer's disease.

Network Pharmacology Evaluation of Tulsi in Alzheimer's Disease

Prediction of Targets linked to Phytochemicals and Alzheimer's Disease

Around 437 targets of all 14 phytocompounds were predicted by Swiss Target Prediction (http://www.swisstargetprediction.ch/) in the same way as (Shamsol Azman et al., 2023) used Swiss Target Prediction to identify possible gene targets for these drugs. The SMILES structures for all the compounds were submitted with the Homo sapiens option enabled. Alzheimer-related target genes were obtained from GeneCards (https://www.genecards.org/) and only those associated with Homo sapiens were kept. Additionally, targets were filtered to include only those with a relevance score higher than 0.9.

Venn Diagram Construction

To illustrate the common targets between phytocompounds and AD, a Venn diagram was created using internet webserver called Venny 2.90 (https://bioinfogp.cnb.csic.es/tools/venny/index2.0.2.html). Among the 77 targets of Alzheimer's disease and 437 targets of phytocompounds, 64 were found to be overlapping**.** A flowchart of the in-silico screening is shown in Figure 1.

Mapping of Protein-Protein Interactions

Online software (https://string-db.org/) was used to construct the interactions between the proteins of phytocompounds and AD. After that, the PPI network was loaded into Cytoscape (https://cytoscape.org/download.html) in order to visualize it. Cytoscape is a free software program that visualizes, models, and analyzes molecular and genetic interaction networks. Cytoscape's core feature allows researchers to determine and study the interconnection of a set of genes or proteins (Yeung et al., 2008). For the selection of hub genes, CytoHuba was used to identify the genes based on topological methods including betweenness centrality, degree centrality and closeness centrality. The phytochemical compound network that targets the top ten target sites was created by (https://cytoscape.org/download.html).

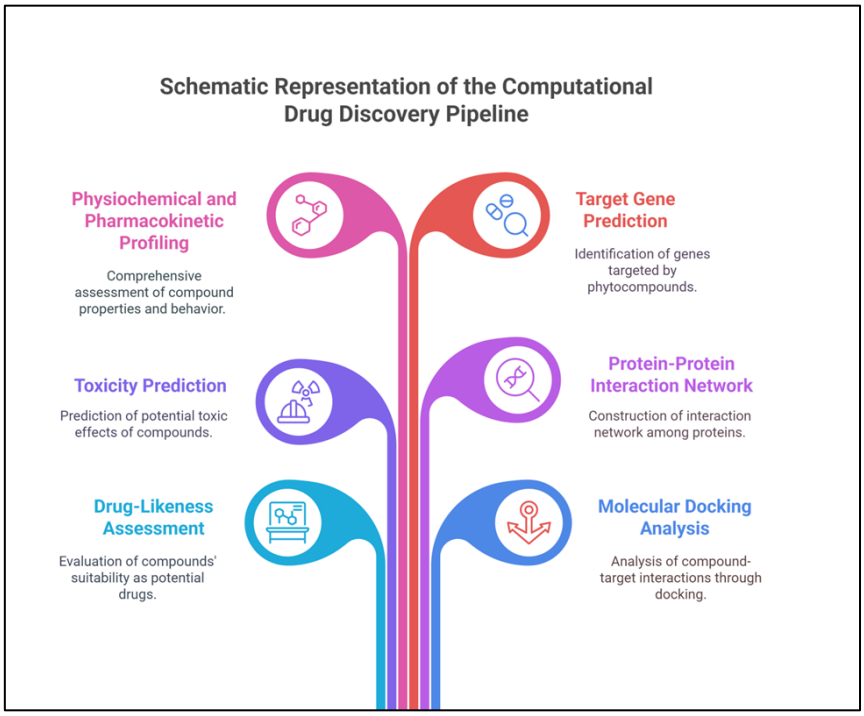


Figure 1: Flowchart of the in-silico screening

Molecular docking Analysis

Ligand Acquisition and Preparation

The scientific literature was examined to identify the bioactive compounds of *O. sanctum*. Chemical structures were downloaded in SDF or SMILES format from PubChem (https://pubchem.ncbi.nlm.nih.gov/). All the structures were then converted into their 3D form by adding hydrogen atoms to complete the molecules. Then, the ligands underwent energy minimization in order to attain stable geometries. Lastly, prepared ligand structures were saved into different formats suitable for further computational analyses, such as target prediction and molecular docking studies.

Protein Acquisition and Preparation

The top five targets identified through CytoHubba scoring were chosen for molecular docking. Their 3D structures were downloaded in PDB format from the Protein Data Bank (https://www.rcsb.org/), as follows: **SRC (ID# 1Y57), PTGS2 (ID# 5F19), ESR1 (ID# 1A52), MAPK3 (ID# 2ZOQ), GSK3B (ID# 1Q4L).**

Docking Procedure

Molecular docking was performed using PyRx 0.8, which uses AutoDock Vina to predict the interaction of ligands with proteins. Molecular docking is a bioinformatics modeling tool used in structure-based drug development. It has been an invaluable resource for drug discovery and biological system research. It is critical for anticipating the interactions of small molecules (ligands) with a protein of interest's binding site. This contributes to the development of more effective and targeted medications (Ayodele et al., 2023). The prepared ligands and protein targets were imported in PDBQT format. Prior to docking, all water molecules, co-crystallized ligands, and other non-essential heteroatoms were removed from the protein structures, while polar hydrogens were added to ensure proper hydrogen bonding.

A grid box was defined around the active site of each protein to allow the ligands to explore the binding pocket. The default parameters for exhaustiveness were applied, and multiple docking runs were performed to identify the most stable binding conformations. Docking results were analyzed based on binding affinity values in kcal/mol. The best conformations, which showed the lowest binding energies, were selected for further study.

Visualization of Docking Results

After molecular docking was conducted through PyRx, the docked interaction was visualized and analyzed through computational based software, Discovery Studio (https://discover.3ds.com/discovery-studio-visualizer-download). It was employed to access the type of bonds between the targets and compounds as well as the bond distance between these interactions.

RESULTS:

Phytochemical Compounds and Synthetic Drugs

The following table includes the molecular and structural characteristics of both phytocompounds and synthetic drugs. This data is compiled from **PubChem** (https://pubchem.ncbi.nlm.nih.gov).

Table 1: The phytocompounds and synthetic drugs, their chemical formula and SMILES taken from PubChem

Compound Name	Code	Chemical Formula	SMILES	PubChem CID	References
Apigenin	P1	$C_{15}H_{10}O_5$	C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O	5280443	Hasan et al., 2023 & Bhattacharjya et al., 2019
Luteolin	P2	$C_{15}H_{10}O_6$	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O	5280445	
Orientin	P3	$C_{21}H_{20}O_{11}$	C1=CC(=C(C=C1C2=CC(=O)C3=C(O2)C(=C(C=C3O)O)[C@H]4[C@@H]([C@H]([C@@H]([C@H](O4)CO)O)O)O)O)O	5281675	
Vitexin	P4	$C_{21}H_{20}O_{10}$	C1=CC(=CC=C1C2=CC(=O)C3=C(O2)C(=C(C=C3O)O)[C@H]4[C@@H]([C@H]([C@@H]([C@H](O4)CO)O)O)O)O	5280441	
Rosmarinic acid	P5	$C_{18}H_{16}O_8$	C1=CC(=C(C=C1C[C@H](C(=O)O)OC(=O)/C=C/C2=CC(=C(C=C2)O)O)O)O	5315615	
Caffeic acid	P6	$C_9H_8O_4$	C1=CC(=C(C=C1/C=C/C(=O)O)O)O	689043	
Ursolic acid	P7	$C_{30}H_{48}O_3$	C[C@@H]1CC[C@@]2(CC[C@@]3(C(=CC[C@H]4[C@]3(CC[C@@H]5[C@@]4(CC[C@@H](C5(C)C)O)C)C)[C@@H]2[C@H]1C)C)C(=O)O	64945	
Linalool	P8	$C_{10}H_{18}O$	CC(=CCCC(C)(C=C)O)C	6549	

Eugenol	P9	$C_{10}H_{12}O_2$	COC1=C(C=CC(=C1)CC=C)O	3314	
Carvacrol	P10	C10H14O	CC1=C(C=C(C=C1)C(C)C)O	10364	
Donepezil	P11	$C_{24}H_{29}NO_3$	COC1=C(C=C2C(=C1)CC(C2=O)CC3CCN(CC3)CC4=CC=CC=C4)OC	3152	
Rivastigmine	P12	$C_{14}H_{22}N_2O_2$	CCN(C)C(=O)OC1=CC=CC(=C1)[C@H](C)N(C)C	77991	
Galantamine	P13	$C_{17}H_{21}NO_3$	CN1CC[C@@]23C=C[C@@H](C[C@@H]2OC4=C(C=CC(=C34)C1)OC)O	9651	
Memantine	P14	$C_{12}H_{21}N$	CC12CC3CC(C1)(CC(C3)(C2)N)C	4054	

Assessment of Physiochemical Properties and Evaluation of Drug Development Potential
The physiochemical characteristics of the phytochemicals from *O. sanctum,* as shown in Table 2, were analyzed for their potential drug-likeness using the **SwissADME** (http://www.swissadme.ch/). A compound's MW should be less than 500 g/mol for the best oral bioavailability. In this dataset, molecular weight (MW) values ranged from 154.25 g/mol for compound P8 to 456.70 g/mol for P7, indicating favorable molecular size profiles. The topological polar surface area (TPSA) values affecting membrane permeability ranged from 0.00 Å² (P10) to 201.28 Å² (P3), while the rotatable bond count varied from 0.00 (P1, P2, P6) to 1.00 (P14). Molecular refractivity (MR) displayed variation from 47.16 (P6) to 136.91 (P7), suggesting differences in molecular polarizability. Most compounds exhibited optimal lipophilicity for orally active drugs, falling between –0.5 and 5.0. According to Veber's rule, 64.3% of the compounds met criteria for good bioavailability, maintaining fewer than 10 rotatable bonds and TPSA below 140 Å². Additionally, 85.7% of the phytochemicals conformed to Lipinski's Rule of Five, fulfilling molecular weight, lipophilicity, and hydrogen bond criteria essential for oral drug-likeness.

Table 2: Physiochemical properties of phytochemicals derived from *O. Sanctum* and synthetic drugs for Alzheimer's using SWISSADME

Ligands	MW (g/mol)	HA	AHA	FC	RB	HBA	HBD	MR	TPSA (Å²)
P1	270.24	20	16	0.00	1	5	3	73.99	90.90
P2	286.24	21	16	0.00	1	6	4	76.01	111.13
P3	448.38	32	16	0.29	3	11	8	108.63	201.28
P4	432.38	31	16	0.29	3	10	7	106.61	181.05
P5	360.31	26	12	0.11	7	8	5	91.40	144.52
P6	180.16	13	6	0.00	2	4	3	47.16	77.76
P7	456.70	33	0	0.90	1	3	2	136.91	57.53
P8	154.25	11	0	0.60	4	1	1	50.44	20.23
P9	164.20	12	6	0.20	3	2	1	49.06	29.46
P10	150.22	15	0	0.73	0	0	0	68.78	0.00
P11	379.49	28	12	0.46	6	4	0	115.31	38.77
P12	250.34	18	6	0.50	6	3	0	73.12	32.78
P13	287.35	21	6	0.53	1	4	1	84.05	41.93
P14	179.3	13	0	1.00	0	1	1	55.68	26.02

MR: molar refractivity, MW = molecular weight, HA = heteroatoms, AHA = number of aromatic heavy atoms, HA= number of heavy atom FC = fraction CSP RB = rotatable bonds HBA = number of hydrogen acceptors HBDD/HBD: Number of Hydrogen Bond Donors TPSA (Topological Polar Surface Area) MR (Molar Refractivity

Table 3: Lipophilicity parameters of phytocompounds and synthetic drugs

Ligands	ILOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT
P1	*1.89*	3.02	2.58	0.52	2.52
P2	*1.86*	2.53	2.28	-0.03	2.03
P3	*1.00*	-0.15	-0.53	-2.51	-0.14
P4	1.63	0.21	-0.23	-2.02	0.33
P5	1.48	2.36	1.65	0.90	1.50
P6	0.97	1.15	1.09	0.07	0.75

P7	3.95	7.34	7.09	5.82	5.46
P8	2.70	2.97	2.67	2.59	2.35
P9	2.37	2.27	2.13	2.01	2.48
P10	3.25	4.38	4.73	4.63	4.19
P11	3.92	4.28	3.83	3.06	4.91
P12	3.20	2.29	2.44	2.34	1.46
P13	2.66	1.84	1.32	1.74	2.03
P14	2.51	3.28	2.69	3.02	2.76

TABLE 4: Evaluation of drug-likeness parameters of phytocompounds and synthetic drugs using SWISSADME

Compounds	LIP	GHO	VEB	EGA	MUEG	BIOSCORE
P1	Yes;0 vio	Yes	Yes	Yes	Yes	0.55
P2	Yes;0 vio	Yes	Yes	Yes	Yes	0.55
P3	No;2 vio	No;1vio	No;1 vio	No;1vio	No;3vio	0.17
P4	Yes;1 vio	Yes	No;1 vio	No;1vio	No;2vio	0.55
P5	Yes;0 vio	Yes	No;1 vio	No;1vio	Yes	0.56
P6	Yes;0 vio	Yes	Yes	Yes	No;1 vio	0.56
P7	Yes;1 vio	No;3vio	Yes	No;1vio	No;1 vio	0.85
P8	Yes;0 vio	No;1vio	Yes	Yes	No;2 vio	0.55
P9	Yes;0 vio	Yes	Yes	Yes	No;1 vio	0.55
P10	Yes;1 vio	Yes	Yes	Yes	No;1 vio	0.55
P11	Yes;0 vio	Yes	Yes	Yes	Yes	0.55
P12	Yes;0 vio	Yes	Yes	Yes	Yes	0.55
P13	Yes;0 vio	Yes	Yes	Yes	Yes	0.55
P14	Yes;0 vio	Yes	Yes	Yes	No;2 vio	0.55

Yes -acceptable, No-not acceptable Vio: violation; (Lipinski rule one violation is considered as acceptable) LIP – Lipinski's Rule of Five GHO-Ghose filter VEB-Veber's rule EGA-Egan Filter MUEG-Muegge filter Bioscore-Bioavailability score

Evaluation of Water Solubility (Log S) of Phytocompounds and Synthetic Drugs using ESOL, ALI, AND SLICOS-IT predictive models

Swiss ADME (http://www.swissadme.ch/) provides three in silico models: Log S ALI, ESOL, SILICOS-IT, for predicting water solubility of the compound studied. ESOL predicted that Compounds P7 (Log S = -7.23; Poorly Soluble) have a solubility of 2.69×10^{-5} mg/ml in water, while P6 (Log S = -1.89; Very Soluble) dissolve to 2.32 mg/ml. Most compounds were predicted as soluble (S). According to the Ali model, P7 would solubilize at 1.92×10^{-6} mg/ml (Log S = -8.38; Poorly Soluble) and P13 would solubilize at 1.31 mg/ml (Log S = -2.34; Soluble). P1, P2, P5, P10, and P11 had a prediction of Moderately Soluble MS, in contrast with the rest that had a Soluble S prediction. For P11 (Log S = –6.90; Poorly Soluble) the solubility values in the SILICOS-IT model were between 4.78×10^{-5} mg/ml and 35.1 mg/ml for C6 (Log S = –0.81; Soluble). Only P11 and P7 were classified under PS (poorly soluble) and MS (moderately soluble), respectively, and most of the compounds fell into the S (soluble) category. P6 was the one with better solubility, again in agreement with that which it showed in the ESOL predictions.

Table 5: EVALUATION OF WATER SOLUBILITY AND SYNTHETIC DRUGS (ESOL, ALI, AND SLICO-IT) OF PHYTOCOMPOUNDS USING SWISSADME

Phytochemical compounds	ESOL				ALI				SILICOS-IT		
	Log S	Solubility		Class	Log S	solubility		class	Log S	Solubility	
		mg/ml	mol/ml			mg/ml	mol/ml			mg/ml	mol/ml
P1	-3.93	3.07e-03	1.13e-03	S	-4.58	6.89e-03	2.54e-05	MS	-4.41	1.06e-02	3.95e-05
P2	-3.72	5.62e-02	1.98e-04	S	-4.52	8.85e-03	3.08e-05	MS	-3.83	4.28e-02	1.50e-03
P3	-2.71	9.01e-01	2.02e-03	S	-3.61	1.00e-01	2.38e-04	S	-1.78	7.31e+00	1.62e-02

P4	-2.83	6.28e-01	1.45e-03	S	-3.56	1.15e-01	2.69e-04	S	-2.37	1.80e+00	4.20e-03
P5	-3.45	1.310e-01	3.64e-04	S	-5.03	3.31e-03	9.21e-06	MS	-2.16	2.42e+00	6.70e-03
P6	-1.88	2.31e+00	1.28e-02	VS	-2.39	7.54e-01	4.18e-03	S	-0.82	3.52e+01	1.95e-01
P7	-7.22	2.68e-05	5.88e-08	PS	-8.37	1.91e-06	4.22e-09	PS	-5.68	9.71e-04	2.13e-06
P8	-2.40	6.08e-01	3.96e-03	S	-3.07	1.34e-01	8.74e-04	S	-1.74	2.21e+00	1.42e-02
P9	-2.45	5.68e-01	3.46e-03	S	-2.52	4.91e-01	2.98e-03	S	-2.78	2.65e-01	1.60e-03
P10	-3.86	2.79e-02	1.35e-04	S	-4.11	1.63e-02	8.01e-05	MS	-3.76	3.49e-02	1.70e-04
P11	-4.80	5.86e-03	1.56e-05	MS	-4.80	5.91e-03	1.56e-05	MS	-6.91	4.78e-05	1.25e-07
P12	-2.69	5.18e-01	2.07e-03	S	-2.61	6.06e-01	2.42e-03	S	-3.15	1.76e-01	7.01e-04
P13	-2.93	3.42e-01	1.18e-03	S	-2.33	1.31e+00	4.56e-03	S	-2.96	3.17e-01	1.10e-03
P14	-3.02	1.71e-01	9.58e-04	S	-3.51	5.65e-02	3.15e-04	S	-2.80	2.85e-01	1.59e-03

S: solubility, MS: moderate solubility, PS: poor solubility, VS: very soluble

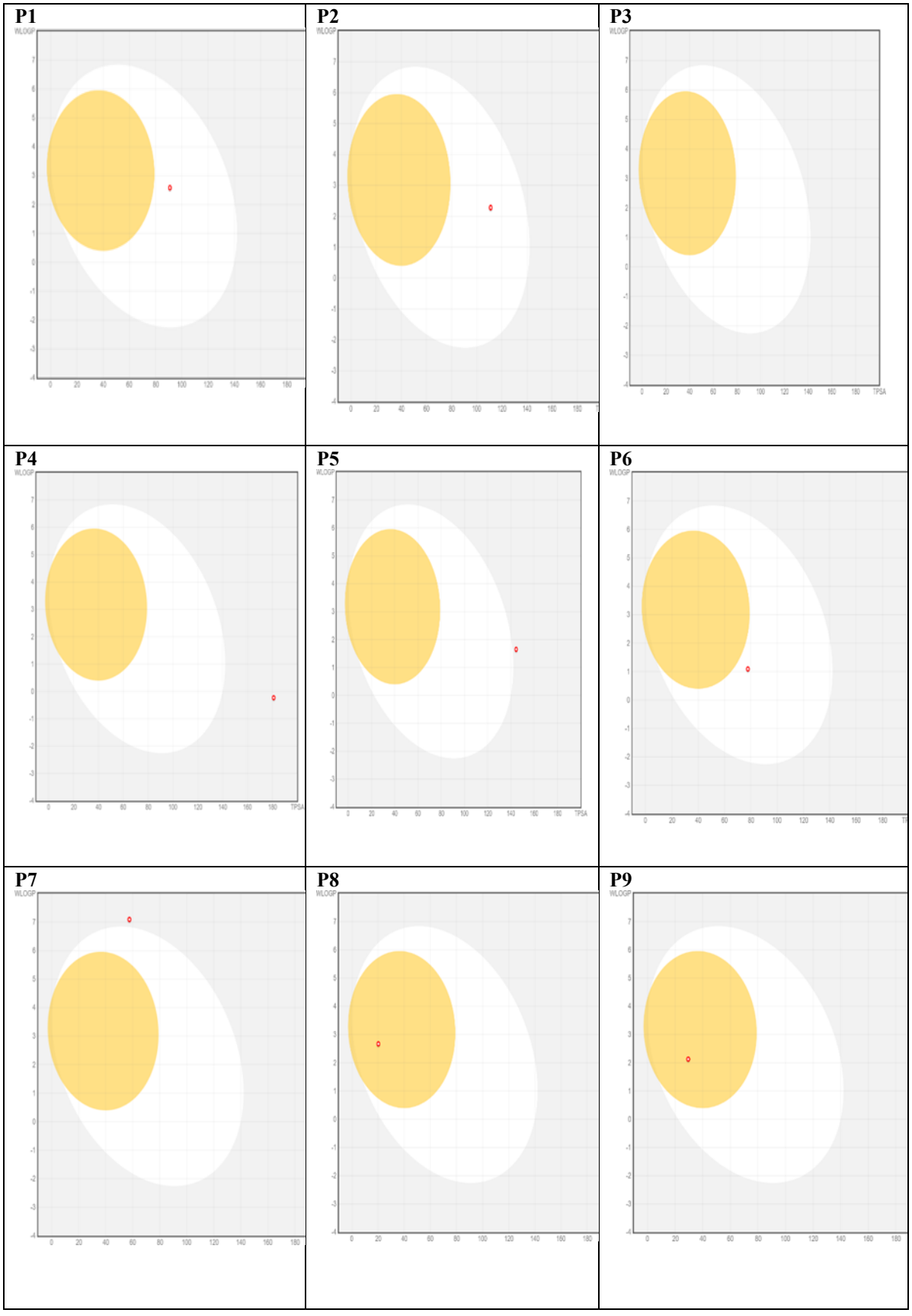
Pharmacokinetic Profiling using ADMET Parameters

The pharmacokinetic features of ten phytocompounds and four synthetic medicines were studied using pkCSM modeling. The results showed that most compounds had high intestinal permeability, ranging from 32.51% to 100%. Caco-2 permeability ranged from −1.25 cm/s to 1.59 cm/s, and water solubility from −5.55 to −2.25 log mol/L. VDss values ranged from -1.08 L/kg to 1.48 L/kg, with BBB permeability ranging from -1.64 to 0.73. Compounds P7, P9, P12, and P14 showed potential for BBB penetration. Metabolic profiling revealed that P11 was the least stable molecule, acting as a substrate and inhibitor of CYP3A4 and CYP2D6, while P1, P2, and P9 inhibited CYP1A2. P11, P13, and P14 showed kidney OCT2 substrate potential. Overall, P7, P9, P12, and P14 were suitable cnadidates for absorption and BBB permeability, but P11 had a high interaction risk due to CYP involvement.

Table 6: Pharmacokinetic profiling using ADMET parameters of common anti-Alzheimer's medications and phytochemicals from *Ocimum Sanctum* using PKCSM

Phytochemicals	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**	**P13**	**P14**
Absorption														
Water solubility	-3.329	-3.094	-2.905	-2.845	-3.059	-2.33	-3.072	-2.612	-2.25	-5.555	-4.648	-2.347	-2.641	-2.317

Caco2 permeability	1.007	0.096	-1.25	-0.956	-0.937	0.634	1.171	1.493	1.559	1.423	1.273	1.569	1.594	1.329
Intestinal absorption (human)	93.25	81.13	43.733	46.695	32.516	69.407	100	93.163	92.041	94.845	93.707	88.456	94.994	91.234
Skin Permeability	-2.735	-2.735	-2.735	-2.735	-2.735	-2.722	-2.735	-1.737	-2.207	-1.58	-2.585	-2.882	-3.75	-2.436
P-glycoprotein substrate	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	Yes	No	No	No
P-glycoprotein I inhibitor	No	No	No	No	No	No	No	No	No	No	Yes	No	No	No
P-glycoprotein II inhibitor	No	No	No	No	No	No	No	No	No	No	Yes	No	No	No
Distribution														
VDss (human)	0.822	1.153	1.486	0.393	-1.088	-1.088	0.152	0.24	0.652	1.266	0.822	0.625	0.89	0.988
Fraction unbound (human)	0.147	0.168	0.258	0.348	0.529	0	0.484	0.251	0.263	0	0.147	0.538	0.36	0.601
BBB permeability	-0.734	-0.907	-1.639	-1.378	-0.647	-0.141	0.598	0.374	0.733	0.157	-0.734	0.508	-0.081	0.603
CNS permeability	-2.061	-2.251	-4.018	-3.347	-2.608	-1.187	-2.339	-2.007	-2.172	-1.464	-2.061	-2.225	-2.511	-2.478
Metabolism														
CYP2D6 substrate	No	No	No	No	No	No	No	No	No	No	Yes	No	No	No
CYP3A4 substrate	No	No	No	No	No	No	Yes	No	No	No	Yes	No	Yes	Yes
CYP1A2 inhibitor	Yes	Yes	No	No	No	No	No	No	Yes	No	No	No	No	No
CYP2C19 inhibitor	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No
Excretion														
Total Clearance (log ml/min/kg)	0.566	0.495	0.374	0.444	0.250	0.508	0.083	0.446	0.282	1.088	0.987	0.557	0.991	0.415
Renal OCT2 substrate	No	No	No	No	No	No	No	No	No	No	Yes	No	Yes	Yes


P1
P2
P3
P4
P5
P6
P7
P8
P9

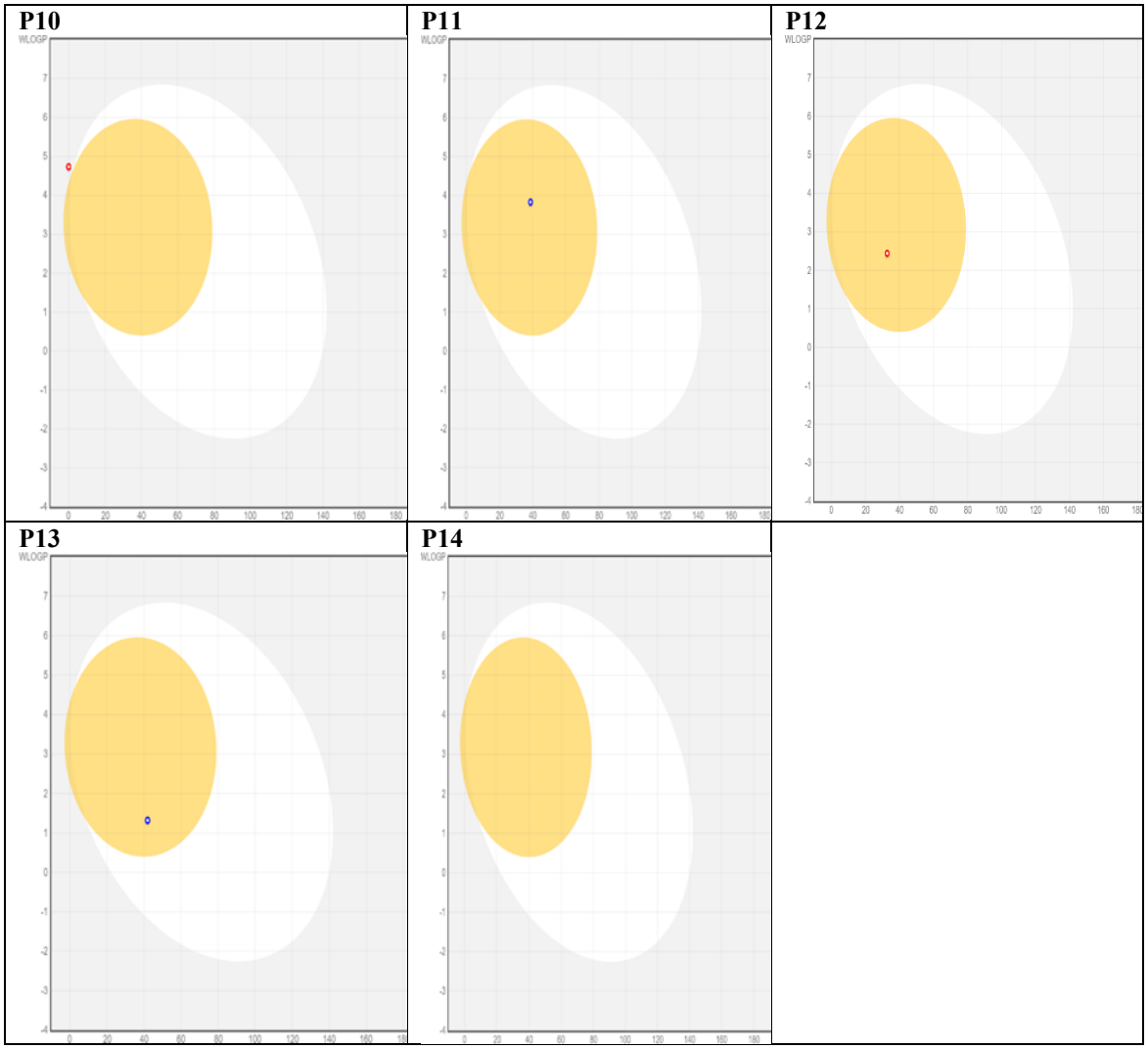


Figure 2: BOILED-Egg model illustrating predicted gastrointestinal absorption (white region) and blood–brain barrier permeation (yellow region) based on WLOGP and TPSA values, generated using SwissADME.

Analysing the Toxicity of Phytocompounds and Synthetic Drugs

The toxicity analysis was conducted by StopTox (https://stoptox.mml.unc.edu) as shown in Table 7. Network string interaction by degree method and their CytoHubba scores are shown in Table 8.

Low-toxicity molecules (P5, P6, P8, P10):

These ligands consistently demonstrated positive margins of safety. StopTox established minimal hazards, with P5 and P10 having low skin and systemic toxicity. These molecules thus are the safest to consider for therapeutic investigation.

Moderate-toxicity compounds (P3, P4, P7, P9, P11, P12):

With LD50 levels 500–2500 mg/kg, these are Class 4 (moderate toxicity). StopTox predictions of this group revealed mixed dermal and oral toxicity trends, although not invariably for all compounds. This indicates these ligands can be further developed but with judicious dose control and toxicity surveillance.

Borderline-safe compounds (P1, P2):

These two compounds are in Class 5 with LD50 values of 2500 mg/kg and 3919 mg/kg respectively, which indicates low toxicity. StopTox analysis indicated skin sensitization potential for both as well. Although less lethal than the moderate group, their risks in immunological and dermal toxicity should not be overlooked.

High-toxicity compounds (P13, P14):

StopTox indicated strong skin sensitization, eye irritation, and acute oral/dermal toxicity for both. Collectively, these findings classify P13 and P14 as unsafe cnadidates with heavy systemic and organ-level toxicity hazards.

Table: 7 Toxicity Evaluation of Phytochemicals and Synthetic drugs using StopTox

Ligands	Skin Sensitization	Acute Inhalation Toxicity	Skin Irritation and Corrosion	Acute Oral Toxicity	Eye Irritation and Corrosion	Acute Dermal Toxicity
P1	S 60%	NT 66.0%	N 70%	NT 73.0%	T 54.0%	T 58.0%
P2	S 70%	NT 68.0%	N 80%	NT 72.0%	T 51.0%	T 64.0%
P3	NS 70%	NT 76.0%	N 80%	NT 75.0%	NT 57.0%	T 57.0%
P4	NS 80%	NT 76.0%	N 80%	NT 75.0%	NT 59.0%	T 57.0%
P5	S 60%	NT 69.0%	N 80%	NT 77.1%	NT 62.0%	NT 63.0%
P6	S 80%	NT 69.0%	N 80%	NT 60%	T 85.0%	NT 54.0%
P7	NS 70%	NT 57.0%	N 60%	NT 88.1%	NT 60.0%	NT 87.0%
P8	NS 80%	NT 57.0%	P 80%	NT 57.0%	T 94.0%	NT 91.0%
P9	S 90%	T 69.0%	N 70%	T 64.0%	T 91.0%	T 59.0%
P10	S 80%	NT 61.0%	P 60%	NT 94.1%	NT 84.1%	NT 79.1%
P11	NS 61%	NT 63.0%	N 80%	T 95.1%	NT 68.1%	NT 66.1%
P12	NS 61%	T 52.0%	N 80%	T 94.1%	NT 61.0%	T 70.0%
P13	NS 80%	NT 56.0%	N 70%	T 91.0%	NT 59.0%	NT 58.0%
P14	S 70%	T 72.0%	P 60%	T 90.0%	NT 83.0%	T 55.0%

Network Pharmacology Analysis of Phytocompounds and Alzheimer's disease

Phytocompounds and Associated Targets

Around 437 targets of all 14 phytocompounds were predicted by Swiss Target Prediction. On the other hand, Gene Card enlisted around 77 targets for Alzheimer's disease and these targets were selected on the basis of having a score of >0.1.

Overlapping Targets between Phytocompounds and Alzheimer disease

To illustrate the common targets between phytocompounds and Alzheimer's disease, a Venn diagram was created. Among the 77 targets of Alzheimer's disease and 437 targets of phytocompounds, 64 were found to be overlapping as shown in f'Figure 2. This depicts a potential of therapeutic involvement between the two.

Evaluation of Protein-Protein Interaction (PPI)

A protein-protein network of 64 targets between the phytocompounds and Alzheimer's disease was made using

the STRING software as shown in Figure 3. All the 64 targets were exported to STRING to analyze their network. SRC, PTGS2, ESR1, MAPK3, GSK3B were some of the most vital targets.

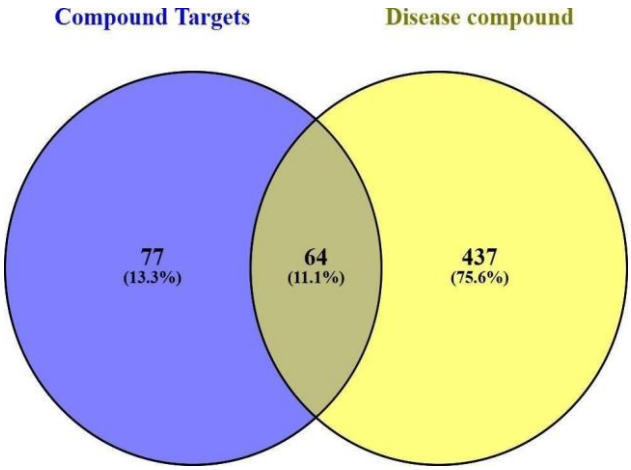


Figure 3: Overlapping targets of 14 phytochemical compounds and Alzheimer disease

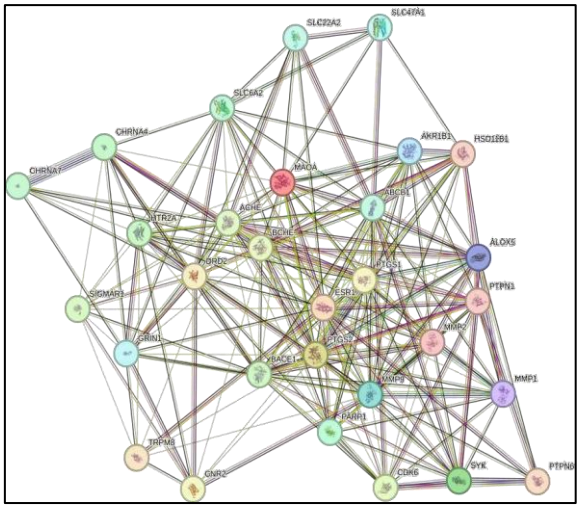


Figure 4: Protein-protein interaction Analysis using STING

Table 8: Top 25 network STRING interaction by degree method and their CYTOHUBA scores

Rank	Name	Score
1	SRC	106
2	PTGS2	84
3	ESR1	78
4	MAPK3	68
5	GSK3B	64
6	MMP9	64
7	MAOA	64
8	DRD2	60
9	MAPK1	54
10	SLC6A3	50

11	NR3C1	50
12	SLC6A4	50
13	APP	48
14	MMP2	46
15	PPARA	44
16	OPRM1	44
17	CHRNA4	44
18	HTR2A	44
19	PARP1	42
20	PIK3CA	42
21	FYN	40
22	ABCB1	40
23	AR	40
24	ACHE	38
25	HDAC1	38

Molecular Docking Analysis

Molecular docking studies of the selected phytocompounds isolated from Tulsi and AD targets were performed to provide a thorough understanding of their binding affinity to the targets. The binding affinity of the phytocompounds was calculated using PyRx molecular docking software in kcal/mol as shown in table 9. Lower docking scores indicate stronger binding and interaction. The binding of 14 ligands (P1 to P14) to five Alzheimer's targets (GSK3B, SRC, PTGS2, ESR1, and MAPK3) was investigated via a molecular docking study.

GSK3B is a principal tau hyperphosphorylation kinase involved in neurodegeneration, and it gave the highest docking score for P1 (-9.2 kcal/mol) indicating a strong and stable binding with the target protein active site. Other good compound series included CP and P4 (-8.3), as well as P2 (-8.2). These compounds could form hydrogen bonds, hydrophobic interactions and π-related interactions, thus contributing to the stability of the ligand in the ATP binding site. P11 (-8.0) and P13 (-7.2) had moderate interactions, P8 (-5.0) was the weakest binder. These observations suggest P1 is the strongest GSK3B binder. P1 might act as an inhibitor of tau pathways in AD.

The SRC kinase has been implicated in synaptic dysfunction in AD. A docking study showed P7 bound to SRC the strongest (-9.8 kcal/mol) among the compounds analyzed. P3 and P4 followed this (both -8.5 and -8.4, respectively), which also featured stable π-alkyl and hydrogen-bonding interactions. P2 (-7.7) and P1 (-7.5) showed good affinity and those of P8 (- 4.0) and P9 (-5.1) showed milder contact with the receptor. An impressive P7 score implies a strong binding affinity between the compound and SRC that may result in modulating synaptic signaling.

PTGS2 is one of the proteins involved in neuroinflammation and in docking experiments P7 had the highest binding energy (-10.8 kcal/mol) among the five proteins tested. P2 and P3 had excellent scores of -9.4, and P1 scored -9.2. Multiple hydrogen bonds and hydrophobic interactions may provide a useful method of inhibition. The binding affinities were predicted to be moderate for both P11 (-8.5) and P4 (-8.7) and weak for P8 (-5.1) and P12 (-5.6). Overall, P7 exhibited highly optimized PTGS2 binding, making it an excellent candidate for Alzheimer's anti- inflammatory therapy.

ESR1 is associated with neuroprotection and cognition. P7 had similar binding affinity (- 8.7 kcal/mol) to P13 (-8.7 kcal/mol). This suggests a role for neuroprotective estrogen-modulatory effects. P4 and P3 also had high affinities of -7.9 and -7.8, respectively, indicating favorable binding stability. P11 (-7.2) and P1 (-7.4) were the

moderate, whereas P8 (-5.0) and P9 (-5.9) were the weakest. These findings suggest P7 and P13 are the best ESR1 interactors for neuronal survival in SH-SY5Y cells.

The main regulator, MAPK3, another protein involved in signal transduction and neuroinflammation, was also tested, yielding the strongest binding energy in the case of P7 (-9.1 kcal/mol), followed by P3 and P11 (-8.9 and -8.8 kcal/mol). Additionally, P4 (-8.6) and P2 (-8.2) showed strong interactions. However, the P8 (-5.3) and P9 (-5.1) interactions were weaker. Furthermore, P7 showed a strong constant score across MAPK3 and other targets, suggesting its multi-targeting potential.

The docking analysis indicates that P7 is the most potent Tulsi phytocompound, which has excellent multi-target inhibitory potential against all the major and pertinent Alzheimer's disease proteins, especially PTGS2 and SRC. Compounds P1, P2, P3, P11 and P13 also showed strong binding interactions which may be related to their known neuroprotective and anti-inflammatory activities.

Table 9: Docking results of phytochemical compounds using PyRx

Compound	**Binding Affinity**
Docking Results of Phytocompounds and Standard Drugs with GSK3B	
P1	-9.2
P2	-8.2
P3	-8.3
P4	-8.3
P5	-7.9
P6	-6.2
P7	-9.0
P8	-5.0
P9	-5.9
P10	-6.6
P11	-8.0
P12	-6.3
P13	-7.2
P14	-6.6
Docking Results of Phytocompounds and Standard Drugs with SRC	
P1	-7.5
P2	-7.7
P3	-8.5
P4	-8.4
P5	-6.6
P6	-6
P7	-9.8
P8	-4

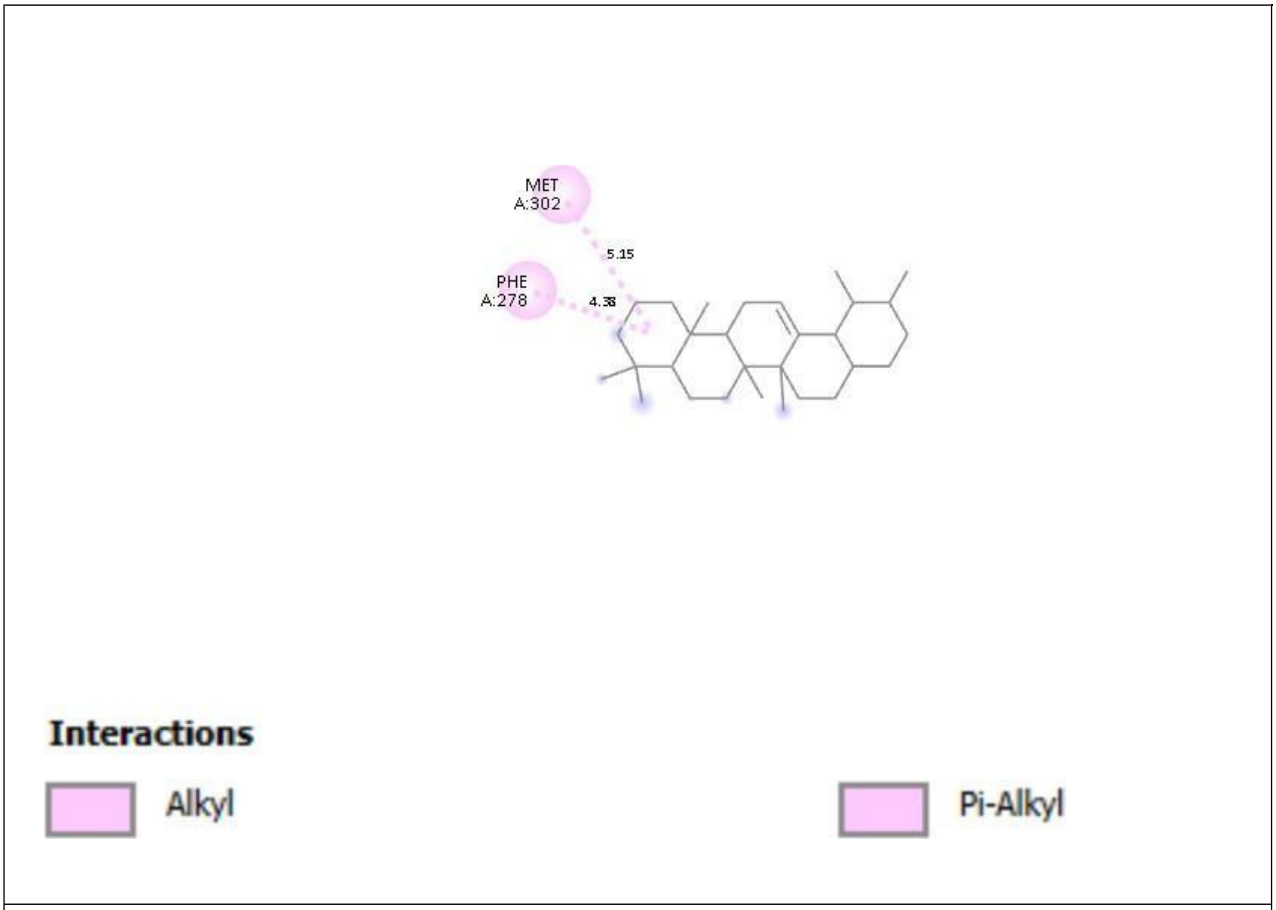
P9	-5.1
P10	-6.7
P11	-7.2
P12	-5.6
P13	-7.1
P14	-5.7
Docking Results of Phytocompounds and Standard Drugs with PTGS2	
P1	-9.2
P2	-9.4
P3	-9.4
P4	-8.7
P5	-7.9
P6	-6.6
P7	-10.8
P8	-5.1
P9	-6.5
P10	-6.7
P11	-8.5
P12	-5.6
P13	-7.8
P14	-6.1
Docking Results of Phytocompounds and Standard Drugs with ESR1	
P1	-7.4
P2	-7.7
P3	-7.8
P4	-7.9
P5	-7
P6	-6.3
P7	-8.7
P8	-5

P9	-5.9
P10	-7.9
P11	-7.2
P12	-5.5
P13	-8.7
P14	-7.1
Docking Results of Phytocompounds and Standard Drugs with MAPK3	
P1	-7.4
P2	-8.2
P3	-8.9
P4	-8.6
P5	-7.9
P6	-6.1
P7	-9.1
P8	-5.3
P9	-5.1
P10	-6.4
P11	-8.8
P12	-6.1
P13	-7.2
P14	-5.8

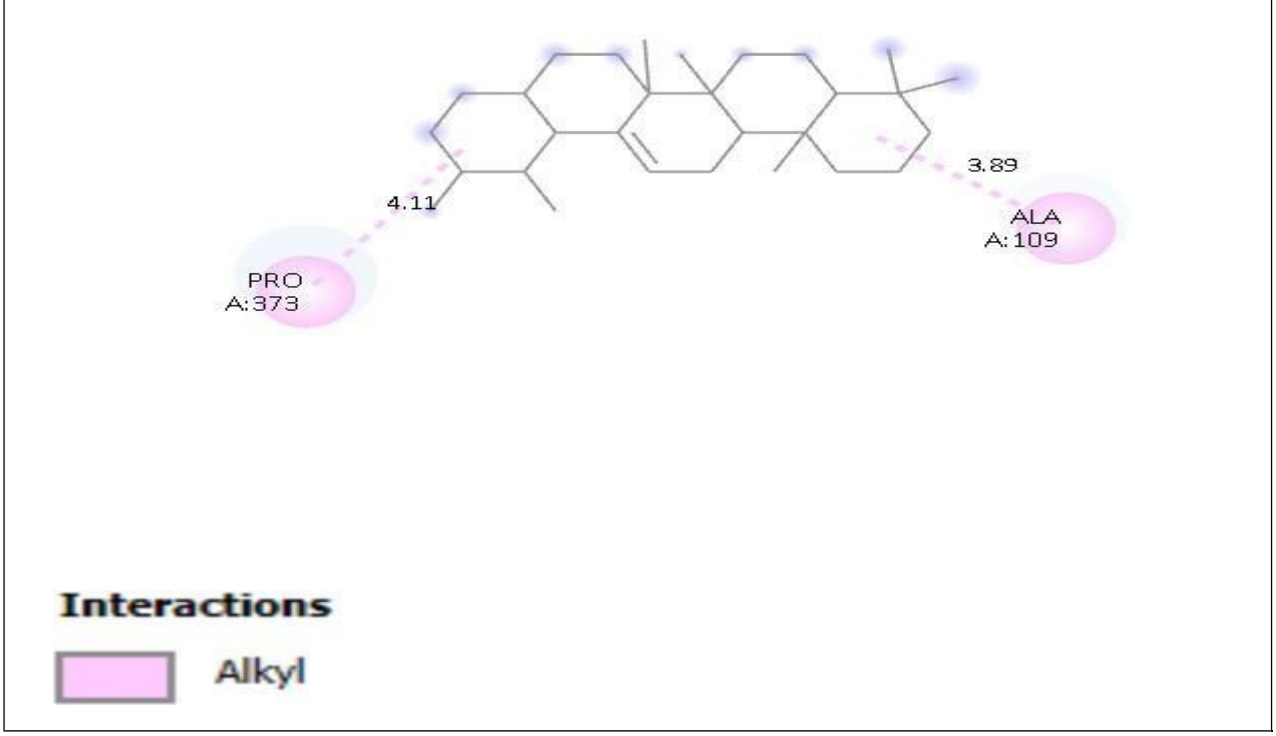
Visualization of Docking Results

After molecular docking was conducted through PyRx, the docked interaction was visualized and analyzed through Computational-Based Software, Discovery Studio (https://discover.3ds.com/discovery-studio-visualizer-download) as shown in Figure 4. It was employed to access the type of bonds between the targets and compounds as well as the bond distance between these interactions.

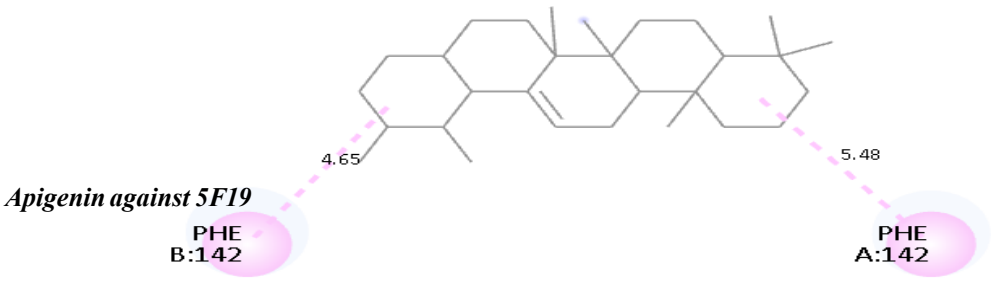
Ursolic acid against 1Y57



Ursolic acid against 2ZOQ



Ursolic acid against 5F19



Apigenin against 5F19

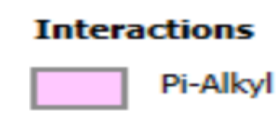
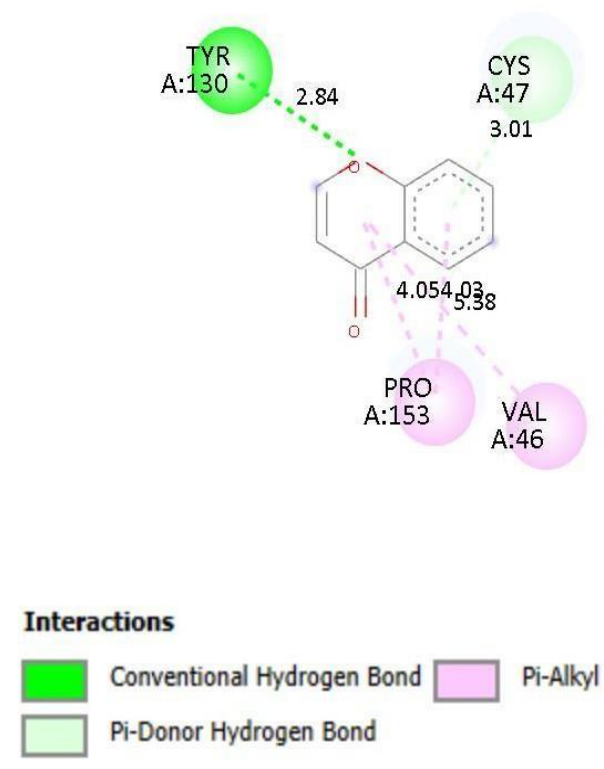


Figure 4: Ursolic acid and Apigenin docked against 1Y57, 2ZOQ, 5F1



DISCUSSION

The results of the current study indicate an enhanced binding capacity of *Ocimum sanctum* phytocompounds towards the major targets of AD GSK3B, SRC, PTGS2, ESR1 and MAPK3. The study also observed that phytocompounds have the potential to target tau hyperphosphorylation, neuroinflammation, synaptic signaling and estrogen mediated neuroprotection. The binding capacity of the phytocompounds towards the major target proteins of AD is an indication of Tulsi constituents as multi-target modulators for inhibiting different AD pathogenesis pathways.

Among all compounds, ursolic acid was found to be the best multi-target compound with the highest binding affinity towards the SRC, PTGS2, ESR1 and MAPK3 protein targets of AD with binding free energy values of -9.8 kcal/mol, -10.8 kcal/mol, -8.7 kcal/mol and -9.1 kcal/mol, respectively. Similar strong binding affinities of structurally similar pentacyclic triterpenoids and ursolic acid analogs with other proteins of AD relating to inflammation and kinases have been reported (Zhang et al., 2022). For example Zhang et al., 2022, stated that on the basis of docking results of PTGS2, hydrophobic and hydrogen-bonding interactions between ursolic acid derivatives and COX-2 (PTGS2) exhibited high docking scores in the range of -9 to -11 kcal/mol (Zhang et al., 2022). Another study by Begum and Veeresh., (2023) reported that the molecular docking simulation revealed strong binding interactions between UA and its derivatives and the active sites of both COX-1 and COX-2 enzymes, suggesting the possibility of dual inhibition. In vitro studies verified the antioxidant properties of these substances, indicating a significant drop in pro-inflammatory mediators and a rise in antioxidant enzyme activities (Begum & Veeresh, 2023).Compounds Apigenin, Luteolin, Orientin, Vitexin, Donepezil, and Galantamine also showed strong binding affinities against a number of targets. For example, -9.2 kcal/mol affinity for the GSK3B target was obtained for the Apigenin ligand, which is in accordance with the earlier docking studies showing the strong inhibition of GSK-3β by several flavonoids. Recent research has identified that epicatechin gallate (-10.93 kcal/mol), Fisetin (-9.44 kcal/mol) and Eriodictyol (-8.54 kcal/mol) exerted higher binding affinities towards the GSK-3β, which is quite consistent with the Apigenin binding in the current work (Viswanathan et al., 2024). GSK3B is a key molecule for tau hyperphosphorylation, the high inhibitory activity of C1 compound also implies possible anti- tau activity.

Compounds Orientin and Vitexin also showed strong binding to all major targets (8.3, 8.9 kcal/mol), which is consistent with recent in-silico studies showing that flavonoids also bind with high affinity and stability to inflammation- or signaling-related proteins. For example, Taherkhani et al. (2023) reported several flavonoids that were found to bind with high affinity to the catalytic domain of MAPK3 (Taherkhani et al., 2023). Flavonoid derivates with high affinity to COX-2 (PTGS2) have been reported by Janakiramulu et al. (2025) that showed stability in molecular dynamics (Janakiramulu & Mamidala, 2025).

The interaction profile of Galantamine showed a high interaction profile with ESR1 (8.7 kcal/mol), the same as ursolic acid in our docking experiments. In silico screening of ERα's ligand- binding domain (LBD) also showed a docking score of −8.8 for Galantamine and it may bind to the ESR1 receptor (Kantarci-Carsibasi & Girgin, 2025). However, no in vitro or in vivo experimental evidence has been reported to date to verify that Galantamine does indeed modulate ESR1 signaling, and while the above docking study provides a basis for a hypothesized mechanism, it is only preliminary in nature and still warrants further study.

The wide range of binding energies (4.0 to 10.8 kcal/mol) obtained against the targets of interest in the present study was similar to the prior molecular docking studies of herbal medicine against neurodegenerative disease targets. Binding energies ≤7 kcal/mol are shown to be biologically important and are probably linked to the downstream therapeutic effects. According to Rahman et al. (2021), most medicines have binding affinities ranging from -6.1 to -7.0 kcal/mol. This supports the usage of -7 kcal/mol as a reasonable limit for prioritizing hits (Rahman et al., 2021).

Overall, the current study emphasizes C7 as leading candidate with potent inhibition against kinase-mediated tau pathways vs. inflammation cascades and estrogen-based neuroprotective signaling. Compounds C1, C2, C3, C4, C11 and C13 also exhibit favorable interactions illustrating their possible use for synergy or multi-target drug design. Taken together, these results support the therapeutic potential of Tulsi-based bioactives in Alzheimer's disease and offer a foundation for additional in vitro and in vivo validation studies.

CONCLUSION

Among the important deduction of the work, 11 phytocompounds and synthetic drugs were studied for their potential therapeutic effects on key target proteins involved in AD disease progression i.e. SRC, PTGS2, ESR1, MAPK3, GSK3B. Docking studies revealed that among all the phytocompounds, ursolic acid exhibited the highest binding affinity of about -10.8 kcal/mol with PTGS2. Some other compounds, such as P1, P2, P3 and P11 and P13 showed the significant interactions which might have neuroprotective potentials. In general, Tulsi phytochemicals seem to be interesting multireceptors target lead candidates for preventing AD.

Recommendations:

The conclusion brought forth by this study suggests the following recommendations: in vitro analysis must be carried out to experimentally validate the safety profile of the compounds. Moreover, the therapeutic implications of the compounds must also be experimentally conformed through in vitro analysis. In the future clinical trial can be conducted on human subjects and these phytochemical compounds can be administered to evaluate and assess their potential therapeutic implications as an alternate or complementary treatment options to standard treatment

drugs or procedures. Moreover, the studies conducted in the future can explore the exact therapeutic mechanism of action of these phytocompounds against these AD targets. A formulation to enhance the bioavailability of these compounds should be followed with improvements in how they might be used as novel therapy in AD management. And increasing the awareness of how these natural phytocompounds that are easily present in various fruits and vegetables may be used as therapeutic options adjuvant to the current treatment.

LIMITATIONS:

The pharmacokinetic and toxicity assessments were conducted solely through *in silico* methods, which do not fully replicate biological systems necessitating further experimental validation. And only a limited number of the phytochemical compounds were analyzed other compounds may also hold therapeutic promise and need investigation. The absence of in vivo data further limits conclusive insights into the compounds' efficacy and safety under biological conditions.

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Competing Interest

The authors declare they have no conflict of interest.

Author Contributions Statement

S.K. and M.K.S. conceived and designed the study. S.K., A.N., S.B., M.R.T., and A.H. performed the computational experiments and data curation. S.I. and A.A. conducted methodology validation and assisted in data analysis. K.A. contributed to formal analysis and supervised the technical workflow. S.S.H. and N. assisted in interpretation of results and manuscript revision. S.K. drafted the main manuscript text. A.A. prepared the figures and visualization materials. M.K.S. and A.A. supervised the project and critically revised the manuscript for important intellectual content. All authors reviewed and approved the final manuscript.

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