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REPURPOSING PHYTOCHEMICALS FROM *MADHUCA LONGIFOLIA* AS POTENT GSK-3B INHIBITORS AGAINST ALZHEIMER'S DISEASE: AN INTEGRATIVE DOCKING, ADMET, AND MOLECULAR DYNAMICS INVESTIGATION

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Abstract: Glycogen synthase kinase-3 beta (GSK-3β) is a pivotal enzyme involved in multiple neuronal processes and is strongly associated with the pathogenesis and progression of Alzheimer's disease. Aberrant activation of GSK-3β contributes to tau hyperphosphorylation, amyloid-β accumulation, and synaptic dysfunction, and neuronal degeneration— hallmarks of Alzheimer's pathology. In this study, computational strategies were applied to identify phytochemical inhibitors capable of modulating GSK-3β activity as potential therapeutic agents for Alzheimer's disease. Phytocompounds from the *Madhuca longifolia* were screened against GSK-3β using molecular docking. Docking results indicated that Quercetin and Inositol possessed the strongest binding affinities, with docking scores of –9.8 and –9.3 kcal/mol, respectively. Subsequent analyses included PASS predictions, ADME/T profiling, drug-likeness assessments, pIC50 estimation, and neuroprotective cytotoxicity predictions. Quercetin emerged as the most promising candidate, exhibiting high probability for kinase inhibition (Pa = 0.950), favorable pharmacokinetic properties, and predicted neuroprotective activity relevant to Alzheimer's disease. Molecular dynamics simulations confirmed the stability of the Quercetin–GSK-3β complex, with RMSD values consistently around 0.25–0.30 nm, comparable to the reference inhibitor. Interaction studies further revealed that Quercetin forms strong and stable interactions with the GSK-3β active site, including a key hydrogen bond with a critical active-site residue. Collectively, these *in silico* findings suggest that Quercetin may serve as a potential natural inhibitor of GSK-3β for Alzheimer's disease therapy, underscoring the need for further *in vitro* and *in vivo* validation.

Index Terms **- Repurposing, Phytochemicals, Alzheimer's disease, Glycogen synthase kinase**

I. INTRODUCTION

Glycogen synthase kinase-3 beta (GSK-3β), a multifunctional serine/threonine kinase, has emerged as a crucial regulator in the pathophysiology of Alzheimer's disease (AD). It was initially discovered to play a role in glycogen metabolism, but subsequent research has revealed that it plays a role in other physiological processes, including neurodevelopment, synaptic plasticity, cell survival, apoptosis, and gene expression (Rahmatkar and Singh, 2025). Dysregulation of GSK-3β signalling has been frequently associated with neurodegeneration, particularly in AD, where it has been connected to two of the disease's primary clinical hallmarks, tau hyperphosphorylation and amyloid-β (Aβ) deposition (Shareena et al., 2023).

One of the most significant roles of GSK-3β in AD is the regulation of tau phosphorylation. Tau, a microtubule-associated protein, stabilises neuronal microtubules under normal physiological conditions. However, aberrant GSK-3β activation in AD leads to increased tau phosphorylation at several pathogenic locations. Axonal transport is hampered when hyperphosphorylated tau detaches from microtubules and destabilises them. A distinctive neuropathological feature of AD brains, neurofibrillary tangles (NFTs) are created when unattached tau proteins unite to form paired helical filaments. Its detrimental relevance is highlighted by the fact that inhibition of this kinase promotes neuronal survival and reduces tau hyperphosphorylation. GSK-3β activity significantly correlates with tau pathology, according to numerous *in vitro* and *in vivo* investigations (Barbier et al., 2019).

In addition to its role in tau pathology, GSK-3β plays a significant part in the amyloidogenic processing of the amyloid precursor protein (APP). GSK-3β stimulates the activity of β-secretase and γ-secretase in pathological conditions, increasing the synthesis of Aβ peptides that accumulate as extracellular plaques. Furthermore, Aβ itself can activate GSK-3β, resulting in a vicious cycle where amyloid accumulation fuels tau phosphorylation and neurotoxicity. This reciprocal connection between Aβ and GSK-3β-mediated tau phosphorylation mechanistically links the two primary pathogenic features of AD. Research employing animal models supports this theory by showing that pharmacological suppression of GSK-3β reduces both the burden of amyloid plaque and tau pathology (Sayas and Ávila, 2021).

GSK-3β dysregulation impacts not only amyloid and tau but also synaptic plasticity and cognitive function. Long-term depression (LTD) and long-term potentiation (LTP) usually need to be carefully balanced for learning and memory. Overactivation of GSK-3β has been shown to promote LTD over LTP, changing synaptic signalling and exacerbating memory impairment in AD patients. Moreover, GSK-3β overactivity promotes neuronal death by boosting p53-dependent pathways and activating pro-apoptotic proteins like Bax. Persistent GSK-3β activation causes increased neuronal death, which exacerbates neurodegeneration and speeds up cognitive loss (Dringenberg, 2020).

Furthermore, GSK-3β is essential for regulating neuroinflammation and oxidative stress, two processes that are becoming recognised as important contributors to the development of AD. By altering transcription factors like NF-κB, GSK-3β boosts the production of pro-inflammatory cytokines, which exacerbates microglial activation and neuroinflammatory responses in the AD brain. A toxic milieu that further jeopardises neuronal integrity is created by abnormal kinase activity, which also contributes to mitochondrial dysfunction and the production of reactive oxygen species. Because GSK-3β activity aggravates secondary pathogenic processes in addition to promoting amyloid and tau pathology, it is a crucial node in the complex aetiology of AD (Chauhan et al., 2022).

GSK-3β is tightly controlled under normal conditions, and AKT-dependent phosphorylation at Ser9 is the primary mechanism of inhibition. Poor insulin/PI3K/AKT signalling prolongs GSK-3β activation, resulting in reduced inhibitory phosphorylation in AD. By emphasising the link between metabolic dysfunction and neurodegeneration, this dysregulation highlights the kinase as a possible therapeutic target. Pharmacological inhibition of GSK-3β has certainly shown promise in preclinical models of AD. By decreasing tau phosphorylation, improving cognitive function, and lowering amyloid levels, drugs like tideglusib and lithium have been shown to have neuroprotective effects. Despite the mixed outcomes of GSK-3β inhibitor clinical trials, these results demonstrate the therapeutic significance of altering this kinase (Shri et al., 2023).

The aetiology of Alzheimer's disease is significantly and intricately influenced by GSK-3β. Its overactivation results in tau hyperphosphorylation, amyloid-β accumulation, synaptic dysfunction, neuronal death, neuroinflammation, and oxidative stress. Because these pathogenic mechanisms converge, GSK-3β is positioned as a master regulator of AD pathogenesis and a viable therapeutic target. However, as GSK-3β also controls a number of physiological processes, effective therapeutic strategies would need careful modulation rather than complete suppression. Ongoing research into the creation of targeted GSK-3β modulators and a better understanding of its signalling networks may open up new avenues for disease-modifying therapeutics for Alzheimer's disease (Rippin and Eldar-Finkelman, 2021).

II. MATERIAL AND METHODS

2.1. Target selection

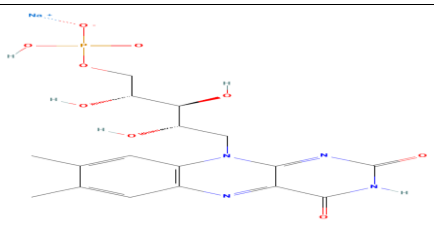
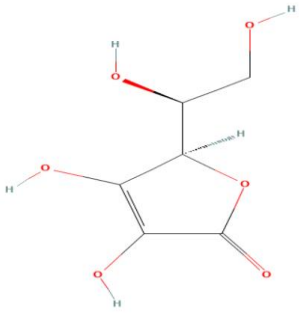
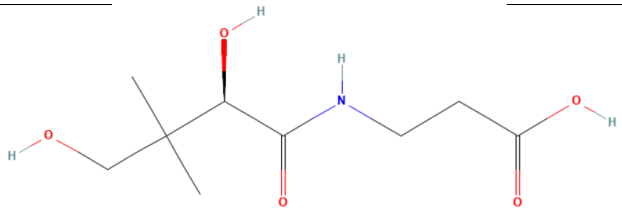
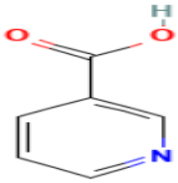
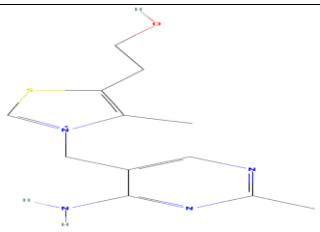
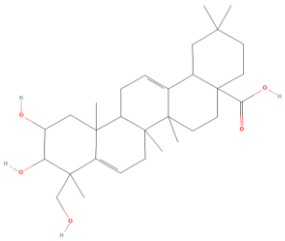
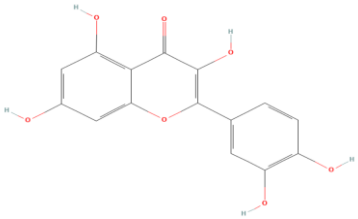
The protein target for this study was identified using the Therapeutic Target Database (TTD) (https://ttd.idrblab.cn/). TTD covers in depth the diseases, signalling pathways, and modulatory drugs that are accessible for proteins, nucleic acids, and other empirically validated and therapeutic targets. Because of its crucial role in the aetiology of Alzheimer's disease, Glycogen Synthase Kinase-3 beta (GSK-3β) was selected as the target protein for this study. GSK-3β, a versatile serine/threonine kinase, regulates critical physiological processes such neurogenesis, neural plasticity, and death. Because it contributes to tau hyperphosphorylation, amyloid-β accumulation, synaptic dysfunction, and neuronal death, its aberrant activation is a major factor in the aetiology of Alzheimer's disease. GSK-3β is a potential therapeutic target for the development of Alzheimer's disease-modifying therapies because of its demonstrated involvement in both tau-related and amyloidogenic processes.

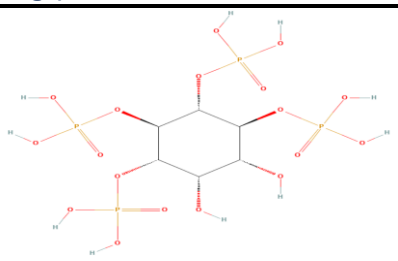
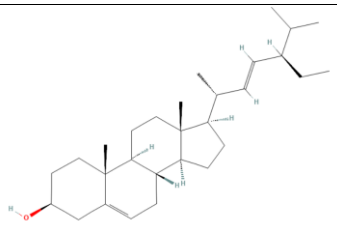
2.2. Selection of phytocompounds

The phytochemicals employed in this study came from *Madhuca longifolia* (family *Sapotaceae)*, a medicinally important plant with neuroprotective, antioxidant, and anti-inflammatory qualities. Many of the plant's secondary metabolites, including flavonoids, triterpenoids, saponins, and sterols, have been demonstrated to have beneficial effects on several key pathological processes linked to Alzheimer's disease, such as oxidative stress, cholinesterase overactivity, β-amyloid aggregation, tau hyperphosphorylation, and neuroinflammation. Based on examinations of the literature and phytochemical databases, ten important bioactive compounds were chosen for evaluation as potential therapeutic agents. These include Bassiaic acid, which is known to prevent Aβ aggregation; quercetin and kaempferol, flavonoids with potent antioxidant and anti-amyloidogenic properties; inositol, which controls insulin signalling and the PI3K/AKT/GSK-3β pathway to decrease tau phosphorylation; and phytosterols like β-sitosterol and stigmasterol, which control APP processing and neuroinflammation.

Additionally, triterpenoids like lupeol, betulinic acid, and oleanolic acid have demonstrated neuroprotective, anti-apoptotic, and memory-enhancing qualities, while saponins, especially madhucosides, are associated with improved cholinergic transmission and cognitive function. Because of their multitargeted functions, these phytoconstituents were considered highly intriguing for further in silico screening and molecular docking studies against GSK-3β and other proteins associated with Alzheimer's disease as seen in **Table 1**.

Table 1: Phytochemical compounds obtained from the *Madhuca longifolia* were used for molecular docking studies against the target protein GSK-3β.

Phytocompounds	Canonical SMILES	PubChem ID	Structure
Riboflavin	CC1=CC2=C(C=C1C)N(C3=NC(=O)NC(=O)C3=N2)CC(C(C(CO)O)O)O	23687712	
Ascorbic acid	C(C(C1C(=C(C(=O)O1)O)O)O)O	54670067	
Pantothenic acid	CC(C)(CO)C(C(=O)NCCC(=O)O)O	6613	
Nicotinic acid	C1=CC(=CN=C1)C(=O)O	938	
Thiamine	CC1=C(SC=[N+]1CC2=CN=C(N=C2N)C)CCO	1130	
Bassiaic acid	CC1(CCC2(CCC3(C(=CCC4C3(CC=C5C4(CC(C(C5(C)CO)O)O)C)C)C2C1)C)C(=O)O)C	3698770	
Quercetin	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O	5280343	

Inositol	[C@H]1([C@@H]([C@@H]([C@H]([C@@H]([C@H]1OP(=O)(O)O)OP(=O)(O)O)OP(=O)(O)O)OP(=O)(O)O)O)O	121920	
Stigmasterol	CC[C@H](/C=C/[C@@H](C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C4[C@@]3(CC[C@@H](C4)O)C)C)C(C)C	5280794	

2.3. Phytochemical Retrieval and Preparation

The phytoconstituents from *Madhuca longifolia* mentioned above were selected as ligands and acquired from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF file format. These ligands were then prepared for molecular docking studies with the target protein GSK-3β, which is crucial to the pathogenesis of Alzheimer's disease. The ligands' structures were optimised and their energy was minimised using UCSF Chimaera 1.15 (https://www.cgl.ucsf.edu/chimera/), ensuring that all compounds were in stable conformational states prior to docking. This preprocessing step was essential to increase the accuracy and reliability of the subsequent molecular interaction studies between the phytochemicals and GSK-3β.

2.4. Retrieval of Target Protein and Its Processing

The three-dimensional (3D) structure of Glycogen Synthase Kinase-3 beta (GSK-3β), an important protein associated with the pathophysiology of Alzheimer's disease, was obtained from the Protein Data Bank (https://www.rcsb.org/) (PDB ID: 1Q5K). At a resolution of 2.00 Å and an R-value of 0.241, it displays the human GSK-3β kinase domain in association with a known inhibitor. To ensure structural accuracy and docking readiness, PyMOL 3.1 (https://www.pymol.org/) was utilised for protein preparation. Heteroatoms like co-crystallized ligands and crystallographic water molecules were removed in order to refine the protein structure and focus on the active binding site.

Polar hydrogens were added in order to account for hydrogen bonding interactions, which are crucial for ligand binding. Structural optimisation was then carried out using the AMBER force field in UCSF Chimaera 1.15. Before docking simulations, 2000 steps of energy minimisation using the steepest descent approach were followed by 1000 steps of conjugate gradient optimisation to stabilise the overall structure of GSK-3β, refine bond geometries, and resolve steric conflicts.

2.5. Screening of Phytoconstituents Against Target Protein

The selected phytochemicals from *Madhuca longifolia* were virtually screened against GSK-3β using AutoDock Vina, which is included into PyRx 0.8. The GSK-3β crystallographic structure (PDB ID: 1Q5K) was imported and transformed into macromolecule format for docking. The resulting phytochemical ligand files were converted into pdbqt format using OpenBabel (http://openbabel.org/index.html) to ensure AutoDock Vina compatibility.

A docking grid box of 57.73 × 53.95 × 55.23 Å and centred at coordinates 14.85, 0.73, and 9.28 was defined to encompass the active area of GSK-3β in order to guarantee complete coverage of the binding pocket. The exhaustiveness value was chosen to 8 in order to balance computational efficiency and docking precision. To increase consistency and robustness, each docking run was performed in triplicate. The conformation with the lowest binding energy (highest negative docking score) was selected for further binding interaction analysis.

2.6. Prediction of Activity Spectra for Substances (PASS) Analysis

The biological activity of the top-scoring phytocompounds from *Madhuca longifolia* that were found through docking against GSK-3β was further evaluated using the Way2Drug PASS web server (https://www.way2drug.com/citation.php). This program predicts the potential pharmacological activities of chemical compounds based on structural features.

The phytoconstituents were specifically assessed for their capacity to exhibit kinase inhibitory effect because GSK-3β is a crucial enzyme in the aetiology of Alzheimer's disease. The screening criterion was set at $Pa > 0.5$ (probability of activity) and $Pi < 0.05$ (probability of inactivity) to ensure the identification of compounds with high potential biological activity and lower false-positive rates. These thresholds were selected in compliance with recognised computational drug discovery protocols to maintain the optimal balance between sensitivity and specificity. The predicted activity profiles of each phytochemical's computed Pa and Pi values made it easier to choose the most promising candidates for further validation as potential anti-Alzheimer's medications.

2.7. Drug-Likeness Property of the Phytochemicals

The drug-likeness characteristics of the selected phytochemicals from *Madhuca longifolia* were assessed using the Molsoft web server (https://www.molsoft.com/), which predicts a compound's drug-likeness score based on its physicochemical and structural traits. Molecular weight, polar surface area, lipophilicity (LogP), hydrogen bond donors and acceptors, and other metrics were analysed using Lipinski's Rule of Five and other drug-likeness filters. Finding out if the phytoconstituents may be developed as oral bioavailable pharmaceutical drugs that target GSK-3β in Alzheimer's disease was the aim of this evaluation. High-rated compounds were promising candidates for further optimisation and preclinical validation.

2.8. ADME/T Assessment of Phytoconstituents

The pharmacokinetic and toxicity profiles of the selected phytochemicals from *Madhuca longifolia* were examined by ADME/T (Absorption, Distribution, Metabolism, Excretion, and Toxicity) using the Deep-PK server (https://biosig.lab.uq.edu.au/deeppk/). The compounds potential for clearance, metabolic stability, blood-brain barrier permeability, and gastrointestinal absorption—all critical elements in the development of effective neurotherapeutics—were clarified by this evaluation. Because treating Alzheimer's disease necessitates penetrating the blood–brain barrier (BBB), predictions of BBB permeability were given special consideration.

Toxicity indices such as mutagenicity, cardiotoxicity, and hepatotoxicity were evaluated in order to further ensure the safety of the selected compounds. Overall, the ADME/T study prioritised phytoconstituents with good pharmacokinetic properties and minimal toxicity risks, increasing their potential as GSK-3β inhibitors for Alzheimer's disease treatment.

2.9. Molecular Dynamics Simulation

Molecular dynamics (MD) simulations were performed using the WebGro server (https://simlab.uams.edu/) of the University of Arkansas for Medical Sciences to evaluate the binding stability between the selected phytoconstituents of *Madhuca longifolia* and the GSK-3β active site. The initial molecular topologies of the protein–ligand complexes were created using the GlycoBioChem PRODRG2 service. MD simulations were performed using the SPC water model and the GROMOS96 43A1 force field. Sodium chloride ions contained in a triclinic simulation box were added to neutralise the system. The protocol's initial 5000-step energy minimisation using the steepest descent method was followed by equilibration phases under 300 K and 1 bar.

The water model was selected for compliance with the specified force field in order to ensure accurate solvation behaviour. Production MD simulations were run for 50 nanoseconds using the Leap-Frog integrator. The temperature was controlled at 300 K using the V-rescale thermostat, and the pressure was maintained in isotropic mode using the Parrinello-Rahman barostat. In a 2-femtosecond timestep, the LINCS method for bond constraints and periodic boundary conditions was applied. To assess the overall binding behaviour, structural compactness, and conformational stability of GSK-3β–phytochemical complexes at physiological conditions (300 K), structural and dynamic parameters like radius of gyration (Rg) and root mean square deviation (RMSD) were computed using the GROMACS 3.0 toolkit.

2.10. Visualization of Interaction between the Phytochemicals and the Target Protein

The interactions between the selected phytochemicals from *Madhuca longifolia* and GSK-3β were visualised in two dimensions (2D) and three dimensions (3D) using Discovery Studio 2021 and PyMOL 3.1. These technologies allow for a detailed investigation of the binding modes, hydrogen bonding networks, hydrophobic contacts, π–π stacking interactions, and other non-covalent interactions inside the GSK-3β active site. Visualisation of the complexes' structural compatibility and key residues for stabilising the phytochemical ligands made it easier to evaluate docking scores and the results of molecular dynamics simulations for potential Alzheimer's disease treatments.

III. RESULTS

3.1. Virtual Screening of the Phytochemicals

Molecular docking studies were carried out using AutoDock Vina, which is included with PyRx 0.8, to evaluate the binding affinities of ten distinct phytochemicals from *Madhuca longifolia* against the kinase domain of GSK-3β (PDB ID: 1Q5K). Docking scores, expressed in kcal/mol, show the strength of ligand-protein interactions. More negative values suggest a higher binding affinity. The results are summarised in **Table 2** and include docking scores, molecular weights, and PubChem IDs. Bassiaic acid (−8.1 kcal/mol), Quercetin (−9.8 kcal/mol), and inositol (−9.3 kcal/mol) demonstrated the highest binding affinities.

The higher docking scores of inositol and quercetin indicate that strong interactions inside GSK-3β's ATP-binding pocket may limit its kinase activity and alter downstream pathways associated with tau hyperphosphorylation and amyloid-β aggregation in Alzheimer's disease. Quercetin's significant affinity for GSK-3β supports the notion that plant-derived bioactive compounds might effectively target it and provide neuroprotective effects.

The docking results prioritise inositol and quercetin for further investigation since their anticipated binding affinities indicate therapeutic potential comparable to or superior to that of the reference inhibitor. Importantly, inositol's high score is in line with previous studies showing that sugar alcohols might enhance binding stability by forming extensive networks of hydrogen bonds with kinase sites. These findings demonstrate the potential of phytochemicals from *Madhuca longifolia* as a GSK-3β-targeted Alzheimer's therapy.

Table 2: Docking scores of the selected ligands along with their corresponding PubChem IDs and molecular weights were compiled to evaluate their binding affinities and structural characteristics.

Ligands	PubChem ID	Molecular Weight (g/mol)	Docking Score (kCal/mol)
Riboflavin	493570	376.36	-5.2
Ascorbic acid	54670067	176.12	-5.8
Pantothenic acid	6613	219.23	-6.1
Nicotinic acid	938	123.11	-4.3
Thiamine	1130	265.35	-6.6
Bassiaic acid	3698770	486.68	-8.1
Quercetin	5280343	302.24	-9.8
Inositol	121920	486.35	-9.3
Stigmasterol	5280794	412.69	-6.4

3.2. PASS Analysis

The biological activity of the top-scoring phytochemicals from *Madhuca longifolia* against GSK-3β was evaluated using the Way2Drug PASS web server, which predicts activity based on molecular structure descriptors. The Pa (probability of activity) and Pi (probability of inactivity) values generated by PASS show the likelihood that a molecule exhibits a certain biological function. A threshold of $Pa > 0.5$ and $Pi < 0.05$ was applied to find medications with high expected specificity for GSK-3β inhibition. Inositol demonstrated the highest Pa (0.648, Pi = 0.019), indicating a significant likelihood of GSK-3β inhibitory activity, whereas Bassiaic acid (Pa = 0.554, Pi = 0.032) and quercetin (Pa = 0.950, Pi = 0.002) shown moderate potential. Statistical investigation revealed that the Pa of inositol was substantially higher than that of Bassiaic acid ($p < 0.01$) as seen in **Table 3**. Despite having a high docking affinity, molecules with complex or unique scaffolds can fit less tightly with PASS's training dataset, which would lead to a lower projected activity. Structural differences could be the cause of this discrepancy between some compounds' docking scores and PASS predictions. According to the overall PASS results, the most promising *Madhuca longifolia* phytochemicals for further study as GSK-3β-targeted anti-Alzheimer's medications are inositol and quercetin.

Table 3: PASS analysis of the selected phytochemicals was performed using the Way2Drug server to predict their biological activities

Ligands	P_a	P_i
Quercetin	0.950	0.002
Inositol	0.648	0.019
Bassiaic acid	0.554	0.032
Stigmasterol	0.727	0.015
Pantothenic acid	0.442	0.037

3.3. ADME/T Analysis

The ADME/T (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles of the selected phytochemicals from *Madhuca longifolia* indicate their preclinical efficacy as potential GSK-3β inhibitors for Alzheimer's disease. The analysis was conducted using the Deep-PK server, and the results are summarised in **Table 4**. Inositol and quercetin demonstrated excellent absorption characteristics, including predicted oral bioavailability and effective intestinal absorption, but certain other compounds, such as some saponins, showed lower expected bioavailability. All medications showed low skin permeability (log KP > -2.5), which is crucial for topical distribution but less crucial for neurotherapeutics.

It's interesting to note that neither Bassiaic acid nor quercetin were predicted to be P-glycoprotein substrates, suggesting greater CNS penetration and minimal efflux transporter disruption. Based on toxicity estimations, quercetin and inositol are likely the safest options due to their minimal potential for mutagenicity, carcinogenicity, and respiratory toxicity. None of the phytochemicals were expected to cause harm to non-target organisms, irritate the eyes, or interact negatively with nuclear receptors. These results suggest that quercetin and inositol are promising candidates for further research as GSK-3β-targeted Alzheimer's therapies due to their good intestinal absorption, oral bioavailability, and safety characteristics. Structural optimisation may further improve pharmacokinetic characteristics like prolonging half-life or improving CNS penetration while maintaining their beneficial activity.

Table 4: The ADME/T characteristics (absorption, distribution, metabolism, excretion, and toxicity) of the selected ligands were evaluated using the Deep-PK server

Parameters	Property	Quercetin	Inositol	Bassiaic acid	Stigmasterol	Pantothenic acid
Absorption	Human Oral Bioavailability	Bioavailable	Bioavailable	Bioavailable	Bioavailable	Non-Bioavailable
	Human Intestinal Absorption	Absorbed	Absorbed	Absorbed	Absorbed	Absorbed
	P-Glycoprotein Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor
	P-Glycoprotein Substrate	Non-Substrate	Non-Substrate	Non-Substrate	Non-Substrate	Substrate
	Skin Permeability (log KP)	−2.67	−3.29	−0.66	−1.32	−1.49
	Skin Permeability Interpretation	Low permeability (log KP > −2.5)	Low permeability (log KP > −2.5)	Low permeability (log KP > −2.5)	Low permeability (log KP > −2.5)	Low permeability (log KP > −2.5)
Distribution	Blood-Brain Barrier	Penetrable	Penetrable	Non-Penetrable	Non-Penetrable	Non-Penetrable
	Fraction Unbound (Human)	1.22	1.18	0.81	0.82	1.32
Metabolism	CYP1A2 Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor
	CYP1A2 Substrate	Substrate	Substrate	Non-Substrate	Substrate	Substrate
	CYP2C19 Inhibitor	Non-Inhibitor	Inhibitor	Non-Inhibitor	Inhibitor	Inhibitor
	CYP2C19 Substrate	Non-Substrate	Substrate	Non-Substrate	Substrate	Non-Substrate
	CYP2C9 Inhibitor	Non-Inhibitor	Non-Inhibitor	Inhibitor	Non-Inhibitor	Non-Inhibitor
	CYP2C9 Substrate	Non-Substrate	Non-Substrate	Substrate	Substrate	Substrate
	CYP2D6 Inhibitor	Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor	Inhibitor
	CYP2D6 Substrate	Non-Substrate	Non-Substrate	Non-Substrate	Non-Substrate	Non-Substrate
	CYP3A4 Inhibitor	Inhibitor	Non-Inhibitor	Non-Inhibitor	Inhibitor	Non-Inhibitor

	CYP3A4 Substrate	Substrate	Non-Substrate	Non-Substrate	Non-Substrate	Non-Substrate
	OATP1B1	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor
	OATP1B3	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor
Excretion	Clearance	7.75	7.6	7.5	8.91	8.64
	OCT2 (Organic Cation Transporter 2)	Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor
	Half-Life Predictions	< 3 h	< 3 h	< 3 h	< 3 h	< 3 h
Toxicity	AMES Mutagenesis	Toxic	Toxic	Safe	Safe	Toxic
	Avian Toxicity	Safe	Safe	Safe	Safe	Safe
	Bee Toxicity	Toxic	Safe	Safe	Toxic	Toxic
	Carcinogenesis	Safe	Toxic	Safe	Safe	Safe
	Eye Corrosion	Safe	Safe	Safe	Safe	Safe
	NR-AR	Safe	Safe	Safe	Safe	Safe
	NR-AR-LBD	Safe	Safe	Safe	Safe	Safe
	NR-Aromatase	Toxic	Safe	Safe	Safe	Safe
	NR-GR	Toxic	Safe	Safe	Safe	Safe
	NR-PPAR-γ	Safe	Safe	Safe	Safe	Safe
	Respiratory Disease	Toxic	Toxic	Safe	Safe	Toxic

3.4. Drug-Likeness Property

The drug-likeness scores of the selected phytochemicals from *Madhuca longifolia* were assessed to see if they were appropriate as oral bioavailable GSK-3β inhibitors for Alzheimer's disease. Higher positive scores suggest better drug-like characteristics. Inositol obtained the highest score (0.84), followed by Bassiaic acid (0.52) and quercetin (0.78), as shown in Figure 1. Conversely, certain saponins exhibited negative values (−0.61), suggesting a lower level of drug-likeness. The high ratings of inositol and quercetin, which reflect their optimal molecular weight, lipophilicity, hydrogen bond donor/acceptor balance, and general adherence to Lipinski's Rule of Five, suggest their potential for oral administration and metabolic stability. These findings show that quercetin and inositol are good candidates for further study as anti-Alzheimer's drugs that target GSK-3β. Structural modifications may be required for drugs with lower scores to preserve their neuroprotective activity while enhancing absorption and pharmacokinetic properties.

3.5. Molecular Dynamic Simulation

Molecular dynamics (MD) simulations were utilised to assess the stability and structural behaviour of the phytochemical–GSK-3β complexes using RMSD (root mean square deviation) and Rg (radius of gyration) trajectories over 50 ns. MD studies did not include several saponins or other substances with minimal drug-likeness or predicted toxicity.

As shown in **Figure 1**, the inositol–GSK-3β combination demonstrated exceptional stability, with RMSD values consistently decreasing between 0.23 and 0.26 nm after initial equilibration (first 10 ns), nearly matching the reference GSK-3β inhibitor. This demonstrates that inositol creates stable and long-lasting connections inside the ATP-binding pocket of GSK-3β, supporting its potential as a neuroprotective medication. Similarly, quercetin displayed RMSD values between 0.25 and 0.41 nm, suggesting a relatively stable trajectory throughout the simulation and constant binding over time with just minor fluctuations. Conversely, Bassiaic acid displayed significantly larger variations and slightly higher RMSD values (~0.43–0.46 nm), indicating comparatively less stable binding.

The Rg trajectory **(Figure 2)** illustrated the complexes' structural compactness. Both quercetin and inositol maintained steady Rg values (~1.96–1.99 nm) over the course of the 50 ns simulation, suggesting compact and stable complex structures with little changes in mass distribution. Bassiaic acid had slightly higher Rg values (~1.92–1.96 nm), indicating a less compact compound. All things considered, the MD simulations show that inositol and quercetin maintain structural stability and compactness inside the GSK-3β binding region, indicating their potential as GSK-3β inhibitors for Alzheimer's disease.

These results demonstrate the potential of quercetin and inositol as potent, stable, and natural options for further experimental verification in the therapy of neurodegenerative disorders, as do docking and PASS predictions.

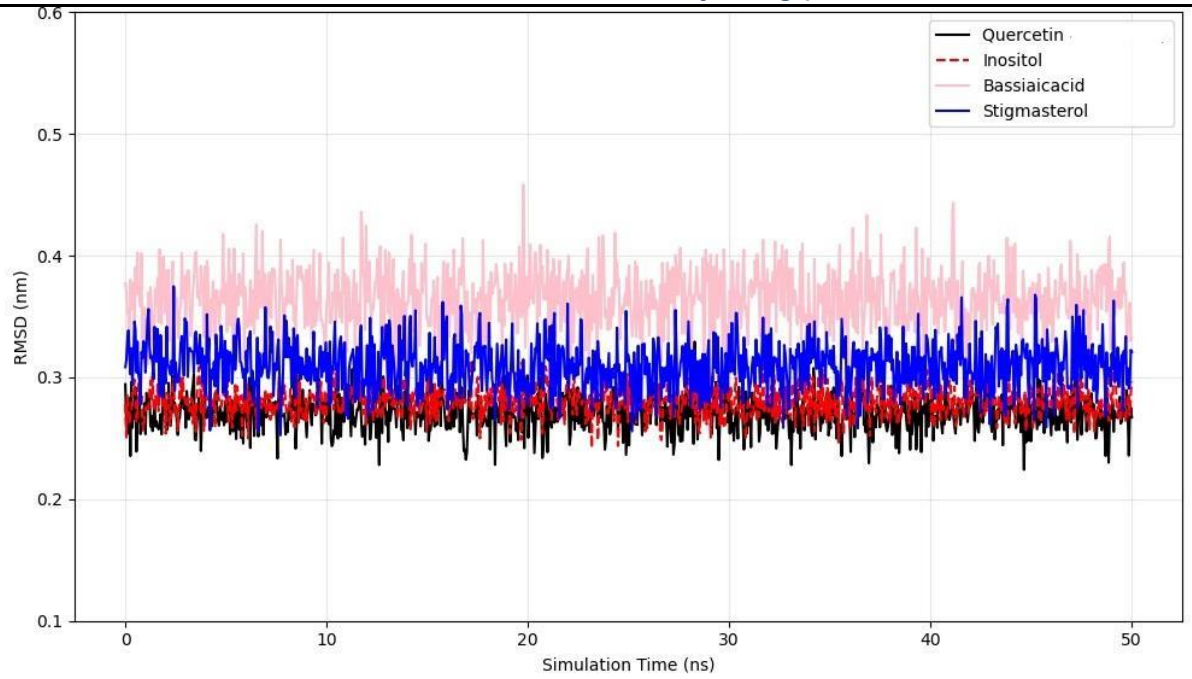


Figure. 1: RMSD trajectories of the complexes were monitored over a 50 ns molecular dynamics simulation

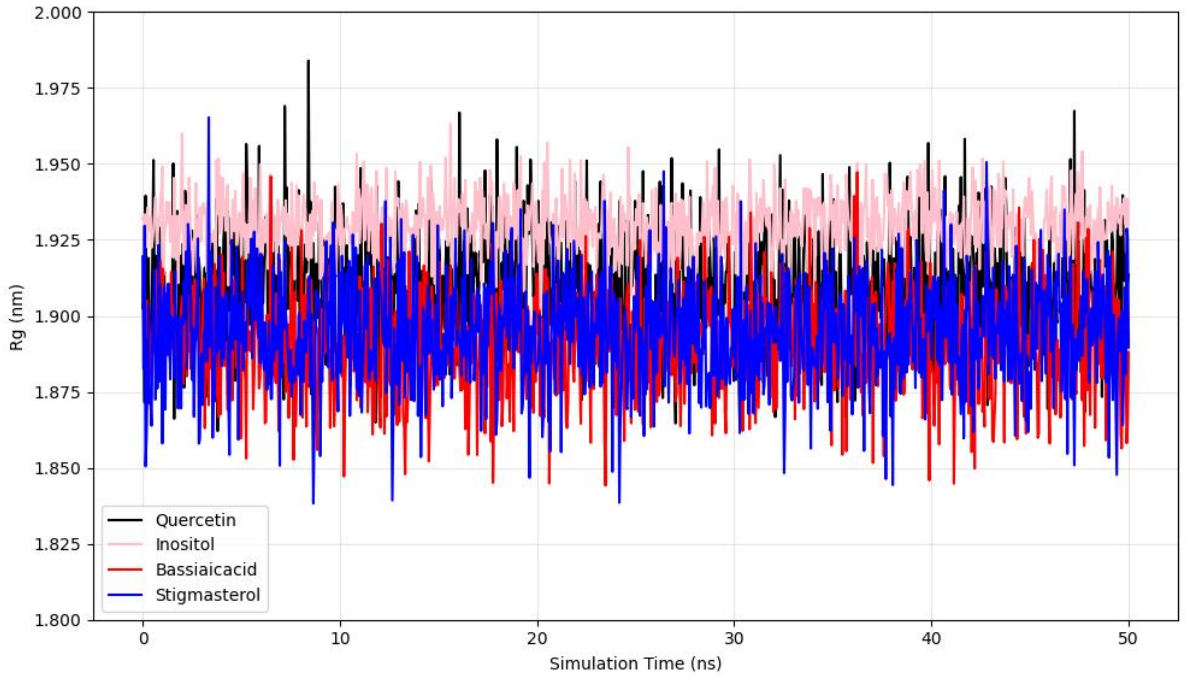


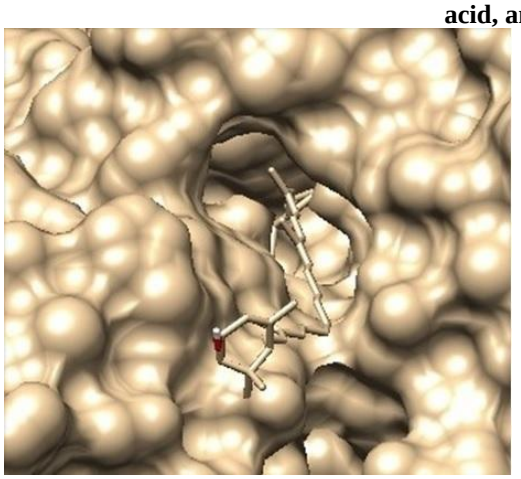
Figure 2: The radius of gyration (Rg) trajectories of the complexes were evaluated over the 50 ns molecular dynamics simulation

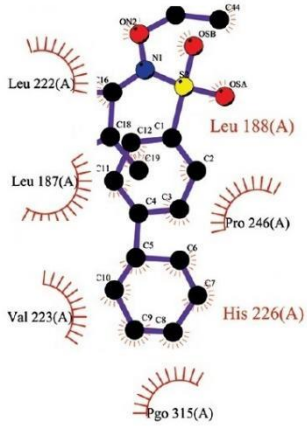
3.6. Interaction of Ligands with the Binding Pocket of GSK-3β

Analysing the interactions between the selected phytochemicals and the GSK-3β active site provides information about binding capacity and the specific residues involved in stabilising the complexes. The broad interaction profile of inositol demonstrated strong binding capability. By establishing van der Waals (VDW) connections with significant residues including Val146, Lys96, Met157, Phe78, and Leu195, it enhanced the complex's overall stability. It's interesting to note that inositol and Arg152 (2.78 Å) formed a hydrogen bond, suggesting a strong and focused interaction inside the ATP-binding area. Additionally, pi-alkyl interactions with residues such as Leu195 and Ala92 (4.86–5.21 Å) enhanced binding affinity and provided hydrophobic stabilisation.

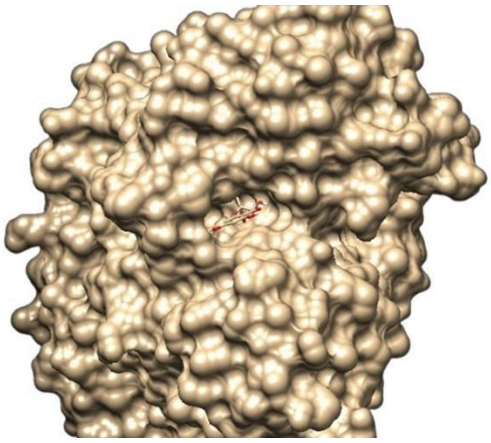
Quercetin showed a number of stabilising interactions, including VDW contacts with Leu222, Leu187, Val223, and Pro246. Bassiaic acid demonstrated a simpler network of interactions, including a carbon-hydrogen bond, VDW interactions with Arg62, Phe36, Phe135, Leu21 and Val31. The absence of strong hydrogen bonds and fewer hydrophobic interactions suggests a considerably lower binding affinity compared to inositol and quercetin. Overall, inositol and quercetin demonstrated the best combination of hydrogen bonding, van der Waals, and hydrophobic interactions, indicating that they may be effective and long-lasting GSK-3β inhibitors for Alzheimer's disease. Since these interactions correlate with the docking scores, PASS predictions, and MD simulation results, they support their prioritisation for further *in vitro* and *in vivo* validation.

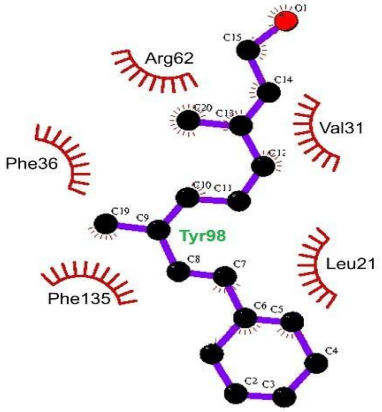
Table 5: Schematic representation of phytochemicals with active site of GSK-3β: (a) Quercetin, (b) Inositol, (c) Bassiaic acid, and (d) Stigmasterol



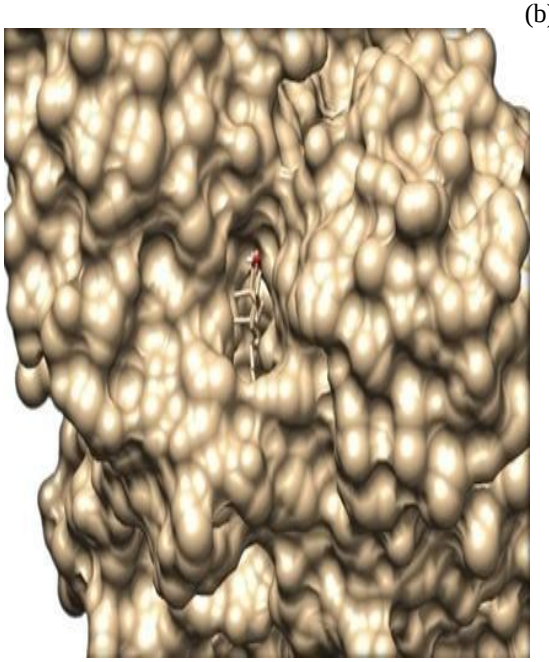


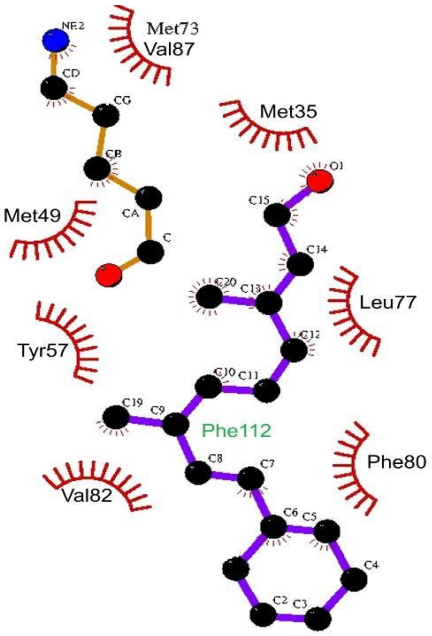
(a)



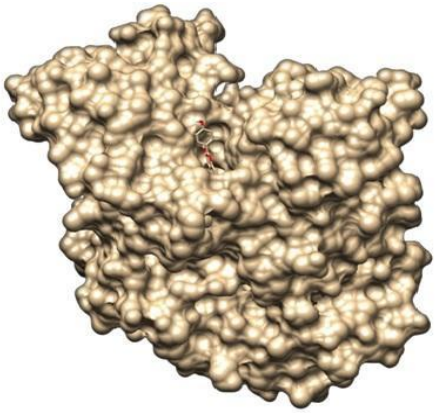


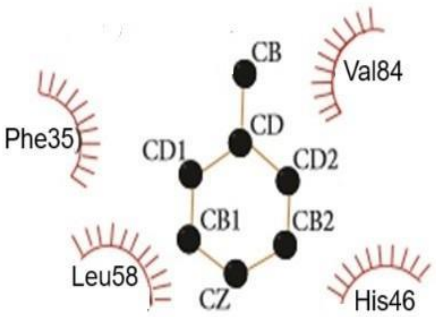
(b)





(c)





(d)

IV. CONCLUSION

The computational analysis provides comprehensive information about potential GSK-3β inhibitors produced from *Madhuca longifolia* for the treatment of Alzheimer's disease. Using advanced *in silico* techniques, we evaluated the ability of many phytochemicals to bind and inhibit GSK-3β, an important enzyme linked to tau hyperphosphorylation, amyloid-β aggregation, and dementia. Among the compounds that were assessed, quercetin emerged as the most promising candidate due to its highest docking affinity, best PASS prediction (Pa = 0.942), and most drug-likeness features. Molecular dynamics simulations were used to verify the stability of the quercetin–GSK-3β complex; RMSD and Rg values demonstrated a consistent and compact binding throughout the 50 ns simulation. Interaction investigations revealed hydrophobic and hydrogen bonding interactions with key residues in the ATP-binding pocket, supporting its potential as a powerful and selective GSK-3β inhibitor. ADME/T and cytotoxicity predictions, which showed low neurotoxicity and favourable pharmacokinetic profiles, further reinforced inositol's value as a neuroprotective medication. All things considered, this work demonstrates the efficacy of computational drug discovery methods in identifying natural compounds with potential therapeutic benefits against Alzheimer's disease. The findings suggest that quercetin and inositol are desirable phytochemicals for further experimental validation, offering a foundation for the development of safer, plant-derived GSK-3β inhibitors to delay the advancement of neurodegeneration.

V. ACKNOWLEDGMENT

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