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Original Article

Multi-Target Mechanistic Insights into *Celastrus paniculatus* against Alzheimer's Disease: Identification of Zeylasteral as a Promising GSK-3β Inhibitor

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ABSTRACT

Background: Alzheimer's Disease (AD), characterized by the accumulation of Amyloid-β (A□) protein, is a progressive neurodegenerative disease. *Celastrus paniculatus* exhibits significant neurological activity, but its exact mechanism in Alzheimer's disease is unknown; consequently, the present study aimed to explore the multiple potential targets involved in AD treatment. **Materials and Methods:** Secondary metabolites of *CP* were identified from the IMPPAT database, and their selection was based on the drug-likeness score. Pharmacokinetic parameters of selected secondary metabolites were determined. Swiss-ADME target predictions were obtained from the Swiss-ADME database, and Venn 2.0 was used to identify the common targets of genes altered in AD and CP. PPI was analyzed using the STRING database, and the SR plot was used for the GO study. **Results:** The current research revealed that 11 selected secondary metabolites exhibit drug-likeness and pharmacokinetic properties. Also, among the 263 common targets, the zeylasteral result identified 89 possible interactions. PPI network of zeylasteral and AD comprised of 89 nodes and 341 edges, with a statistically significant PPI enrichment *p*-value of <1.0e-16, indicating the strong interaction of zeylasteral among the targets. Docking results showed that zeylasteral interacts favourably with GSK-3β, with a docking score of -9.5 kcal/mol and interactions involving key residues: PTR A:216, ARG A:220, ASP B:200, and ASP B:181 of GSK-3B, mitigating AD pathology; however, *in vivo* and dynamic simulation studies are required to confirm this prediction. Discussion: Zeylasteral, a quinone-based natural chemical, was identified as a multi-target agent that modulates AD-relevant signaling pathways, including mTOR, PI3K/Akt, neurotrophin signaling, and autophagy, through the integration of bioinformatics techniques. Zeylasteral is a strong GSK-3B inhibitor, suggesting that it can be used as a lead compound for AD by reducing tau hyperphosphorylation, modulating amyloidogenic APP processing, and restoring synaptic integrity. **Conclusion:** The findings imply that CP demonstrated a strong PPI network connection between CP targets and AD genes via a multi-target, multi-pathway mechanism, suggesting that multiple mechanisms of *Celastrus paniculatus* will contribute to disease prevalence. Future research can investigate the relationship between CP and AD.**Keywords:** Alzheimer's disease, *Celastrus paniculatus*, GSK-3B, Molecular docking, Network pharmacology, Zeylasteral.

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INTRODUCTION

Alzheimer's disease is a neurodegenerative condition characterized by extracellular accumulation of Amyloid-β (A□) protein, neuronal loss, cognitive impairment, and Neurofibrillary Tangles (NFTs).[1-3] It is reported by the World Health Organization (WHO) that, in 2021, approximately 57million people suffered from AD.[4] According to Alzheimer's Disease International, statistics suggest this number will double every 20 years, reaching 78 million in 2030 and 139 million in 2050.[5] Although the pathogenic mechanism is still unknown, it is ultimately the cause of AD. Accumulation of Aβ enhances mTOR signaling, whereas Aβ accumulation can be reduced with decreasing mTOR signaling. The mTOR pathway is crucial for maintaining cellular homeostasis by balancing protein synthesis and degradation. Hyperphosphorylation of microtubule-binding protein Tau, which results in intraneuronal inclusions, Neurofibrillary Tangles (NFTs), and extracellular accumulation of amyloid plaques composed of Amyloid-β peptide, are two major neuropathological contributors to AD.[6] The interference of A□ and tau with distinct signaling cascades can obstruct learning and memory.[7] AD is a multifactorial disease;

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monotherapy has been largely ineffective as it focused only on a single target or mechanism of disease, often due to the interplay of various factors. Therefore, considering the complexity of AD pathogenesis, various multifunctional therapies that exhibit multiple target potential with different mechanisms have been sought for successful treatment, such as N-Methyl-D-Aspartate (NMDA) agonists with Vitamin D. But the long-term efficacy evaluation of combination therapy in slowing cognitive and functional decline in AD compared to monotherapy.[1,8,9] Despite the unprecedented progress made in understanding ADs over the past decade, more basic research remains needed. There is still no efficacious and curative treatment of the disease.[10] Thus, exploring new targets and effective mechanisms for AD and developing preventive and therapeutic interventions is the primary goal of the current research.

Celastrus paniculatus (CP) willd. commonly known as Jyotishmati, is a climbing staff tree belonging to the family Celastraceae.[11,12] In Ayurveda, it is an established medicinal plant recognized for its analgesic, antiepileptic, anti-inflammatory, sedative, anxiolytic, hypolipidemic, anti-spermatogenic, anti-stress, and neuropharmacological effects.[13,14] CP is rich in numerous secondary metabolites, including Alkaloids (Celapanin, Celastrine, Paniculatin), flavonoids (Pristimerin, Lupeol, □-amyrin), sterols (Campesterol, Stigmasterol), fatty acids, and quinones (zeylasteral, celasteral, and zeylasterone).[15,16] *CP* is widely used as folk medicine for neuroprotection and memory enhancement, but the exact mechanism in Alzheimer's disorder is still unknown.[17,18] Alternative therapies have gained popularity due to the limited efficacy of current pharmaceuticals. Quinones, widely distributed in natural plants like *Aloe barbedensis*, Plumbago species, *Rheum emodi* rhizomes, *Embelia ribes* Burm, *Salvia miltiorrhiza*, and *Rubia tinctorum* L., are known to offer potential neuroprotective properties against cognitive decline and AD pathogenesis, which makes them an attractive option.[19] In the context of previous studies, the current work prioritizes zeylasteral as a multi-target compound from *Celastrus paniculatus*, targeting GSK-3B through protein networking and docking analysis.

In the future, further studies will be needed to determine whether other quinones and their derivatives can be used to prevent and treat AD, including investigating additional pathways and genes. With the development of bioinformatics, network pharmacology can systematically explore the drug targets and the underlying molecular mechanisms of complex diseases.

MATERIALS AND METHODS

Screening of *CP* Phytoconstituents

The secondary metabolites of *CP* were retrieved from the Indian Medicinal Plants, Phytochemistry and Therapeutics 2.0 (IMPPAT 2.0) database (https://cb.imsc.res.in/imppat/). The PubChem (https://pubchem.ncbi.nlm.nih.gov/) database was used to obtain the complete chemical profiles of the secondary metabolites of *CP*. The canonical SMILES were used for the estimation of pharmacokinetic parameters of the secondary metabolites by SwissADME (http://www.swissadme.ch/).[20,21]

Determination of the *CP* Targets in AD

Potential targets of secondary metabolites of *CP* were predicted from the Swiss Target Prediction database (SwissTargetPrediction), and the GeneCards database(https://www.genecards.org/).[22,23] was used to identify genes associated with AD. Further analysis was performed by utilizing the UniProt database (https://www.uniprot.org/).[24] The common target genes of *CP* secondary metabolites potentially related to AD were obtained using the VENNY 2.1.0 software (https://bioinfogp.cnb.csic.es/tools/venny/).[25] In order to obtain potential targets for AD, common targets were further analyzed.[8]

Construction of protein-protein interactions and pathways

The potential targets obtained from the selected secondary metabolites of *CP* and the genes altered in AD were introduced into the STRING online database (https://string-db.org/) to construct the protein-protein network between common targets.[26-28]

Enrichment and KEGG pathway analysis

The common targets were used with STRING software for the analysis of Gene Ontology (GO) and KEGG enrichment to screen the biological processes and signaling pathways involved in AD.[29] The results of GO enrichment analysis were plotted using the SR plot online platform (https://www.bioinformatics.com.cn/en).[8]

Molecular docking

Molecular docking verifies the reliability of the screened key genes as receptors and the selected ligand from *CP*. The 3D protein structure of screened AD genes was downloaded from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/), and the zeylasteral 3D conformer in SDF format was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). AutoDock tools, BioVIVA Discovery Studio, and Open Babel are employed for the protein and ligand preparation.[30] PDBQT format of the ligand and protein was utilized for docking analysis in terms of ligand-protein interaction binding energy.[8,31]

Compound toxicity study

The toxicity profile of selected secondary metabolites was determined by using the ProTox 3.0 web server (https://tox.charite.de/protox3/).[32] ProTox 3.0, by using fragment propensity, molecular similarity, and pharmacophore approaches, provides predictions of organ toxicity, acute toxicity, toxicological endpoints, and target toxicity.[33]

RESULTS AND DISCUSSION

Mining of Phytoconstituents present in *CP*

A total of 31 phytoconstituents of the plant *CP* were retrieved from the IMPPAT online database (Table 1). Among these, 13 were selected as unique constituents. The initial examination of phytoconstituents was performed via Swiss ADME analysis for the pharmacokinetic study of constituents which results only 11 compounds including Paniculatadiol, Pristimerin, Tetracosanoic acid, (1S,3S,8S,9R,12S,14R,16S)-16-hydroxy-5,14-dimethyl-5 azatetracyclo [7.7.0.01,12.03,8] hexadecan-10-one, 1-Tetracosanol, Celapanine, Zeylasteral, Celastrol, Zeylasterone, beta-Amyrin, 1-Triacontanol having probability more than 10% and follows Lipinski rule of violation. The PubChem database was used to retrieve properties such as molecular weight, canonical SMILES, and molecular structure, as depicted in Table 1.

Putative targets of the chemical constituents of *CP*

Based on data mining from the online database, 11 unique constituents were identified in the CP. The contribution of individual constituents to the putative target is as follows: Paniculatadiol: 57 targets; Pristimerin: 65 targets; Tetracosanoic acid: 47 targets; (1S,3S,8S,9R,12S,14R,16S)-16-hydroxy-5,14-dimethyl-5 azatetracyclo [7.7.0.01,12.03,8] hexadecan-10-one: 100 targets; 1-Tetracosanol: 19 targets; Celapanine: targets; Zeylasteral: 100 targets; Celastrol: 13 targets; Zeylasterone: 05 targets; beta-Amyrin: 58 targets; 1-Triacontanol: 18 targets. All targets were combined, and after removing duplicates, 291 targets were provided in Figure 1. Similarly, 15507 targets related to AD were retrieved from the GeneCards database. The finalized AD and constituent targets were inserted into Venn 2.1.0 to obtain the common targets. Among these substances, zeylasteral exhibited a high anticipated target count (100 targets) and, crucially, showed 89 shared targets with genes linked to Alzheimer's disease. 89 nodes and 341 edges made up the matching PPI network, which had a strong PPI enrichment ($p<1.0\times10^{-11}$). The zeylasteral-associated targets were implicated in several AD-relevant biological processes and signaling cascades, according to subsequent GO and KEGG enrichment analysis. Zeylasteral was therefore given priority as a representative computational candidate for in-depth docking and network analysis.

Construction of Protein-Protein Interaction (PPI)

The protein-protein interaction network for the 263 common genes of phytoconstituents and AD revealed 263 nodes and 776 edges, with an average degree of 5.9 and a PPI enrichment *p*-value of less than 1.0e-16 (Figure 2a). The PPI network was constructed to determine the potential mechanism of CP constituents for AD. Because zeylasteral was the only constituent highly enriched across many targets, the PPI network of 89 common target genes associated with AD and zeylasteral comprised 89 nodes and 341 edges (Figure 2b), with a statistically significant PPI enrichment *p*-value of less than 1.0e-16, indicating strong interactions among the targets.

Gene ontology and KEGG pathway enrichment analysis

GO and KEGG enrichment analyses were used to identify biological and metabolic pathways that are significantly enriched among intersecting targets. KEGG pathway enrichment analysis revealed that zeylasteral specifically targets 144 pathways, including those related to AD, and also represents 648 biological processes, 69 molecular functions, and 17 cellular components that are significantly enriched. GO results show that peptidyl serine modification and peptidyl serine phosphorylation in biological processes, histone deacetylase complex and glutamatergic synapse in cellular components, and protein serine/threonine kinase activity and protein serine kinase activity in molecular functions are highly enriched, with extremely significant p-values. KEGG analysis revealed that the neurotrophin signaling pathway, sphingolipid signaling pathway, and endocrine resistance are highly enriched, with low *p*-values (Figure 3).

The PPI network between Zeylasteral and AD targets demonstrated a robust connection. Bioinformatics analysis further validated the connection and revealed that zeylasteral acts on APP, Raf, JNK, mTOR, PI3K, GSK-3B, IRE1α, and CKIα (Figure 4).

Molecular docking

The data from the GO and KEGG enrichment analyses were further validated using docking analysis. APP, Raf, JNK, mTOR, PI3K, GSK-3B, IRE1α, and CKIα proteins were targeted with the ligand zeylasteral. All proteins and ligands were prepared using BioVIVA Discovery Studio, Open Babel, and AutoDock Tools, and both were converted to pdbqt format, which is required for AutoDock Vina. The binding interactions of zeylasteral with the target proteins are shown in Table 2. The binding energy for each target protein ranged from -6.0 to -9.5 kcal/mol (Figures 5a-5h). Among all targets, docking analysis of GSK-3β (PDB ID 5K5N), with X, Y, and Z coordinates (11.444180, 14.039140, and 16.847580) of the ligand-binding site within the protein, resulted in strong binding between the ligand and protein, with a binding score of -9.5 kcal/mol. The docking analysis (Figure 5) revealed a multifaceted interaction profile between the ligand and the protein, underscoring the complexity of their binding interface. Notably, the ligand formed 4 conventional hydrogen bonds with key amino acid residues: PTR A:216, ARG A:220, ASP B:181, and ASP B:200, suggesting significant polar interactions that contribute to binding affinity.

Toxicity study

Hepatotoxicity, neurotoxicity, nephrotoxicity, cardiotoxicity, carcinogenicity, mutagenicity, cytotoxicity, ecotoxicity, and clinical toxicity are among the primary toxicity endpoints for which ProTox-3.0 projected zeylasteral to be inactive, as depicted

in Table 3. Nonetheless, it was anticipated that the substance would be active for immunotoxicity, pulmonary toxicity, and nutritional toxicity. It is not advisable to ignore these anticipated positive endpoints since they may be safety flags. Zeylasteral's overall toxicity profile should therefore be regarded as preliminary and needs to be confirmed experimentally.

DISCUSSION

The research on natural products in the context of AD using *in silico* tools provides new avenues in neurodegenerative disease research.[32]AD is a serious multifactorial disease and poses challenges in determining the etiology, pathogenesis, and impeding the advancement of the illness.[34] The progress of the disease cannot be fundamentally delayed with the single-target acting drugs, as far as these drugs could only relieve the symptoms of AD due to its multifactorial origin.[35] For instance, AChE inhibitors (rivastigmine, galantamine, and donepezil) were the first FDA-approved anti-Alzheimer drugs, which are believed to elevate the cholinergic level by inhibiting the AChE enzyme without significantly affecting the disease progression.[36,37] Combination of two single-target drugs has been used for AD treatment, but this may lead to an increased risk of drug resistance and adverse effects.[35,38] The network pharmacology approach enhances the understanding of AD's pathological mechanism by means of various targets and mechanisms, as single-targeted drugs are less efficient in AD's treatment. As quinone-based compounds are the principal treatment option for AD, Zeylasteral is a promising candidate for therapeutic use in Alzheimer's Disease (AD).[39] In this research, integrated strategy of network pharmacology which introduced data mining, target prediction, common target assessment, enrichment analysis, PPI study and docking was used to explore the potential target and therapeutic mechanism of zeylasteral in AD.

Different research demonstrated that mTOR activation enhances A□ accumulation and deposition by modulating the metabolism of APP protein and also contributes to the phosphorylation of Tau protein. Regulation of mTOR through several signaling pathways, PI3K/Akt, GSK-3, AMPK, and IGF-1 helps to regulate A□ clearance.[40]

The analysis of *CP* by network pharmacology revealed 11 selected secondary metabolites and 296 common targets with AD, indicating multi-target modulatory action. The Protein-Protein Interaction (PPI) network indicated that CP-related targets are highly involved in AD pathology, and KEGG pathway analysis and GO enrichment analysis further demonstrated that the targets are involved in major signaling cascades such as neurotrophin signaling, oxidative stress regulation, and autophagy. Notably, Zeylasteral was the only bioactive component consistently enriched in AD-relevant pathways, supporting its therapeutic importance.

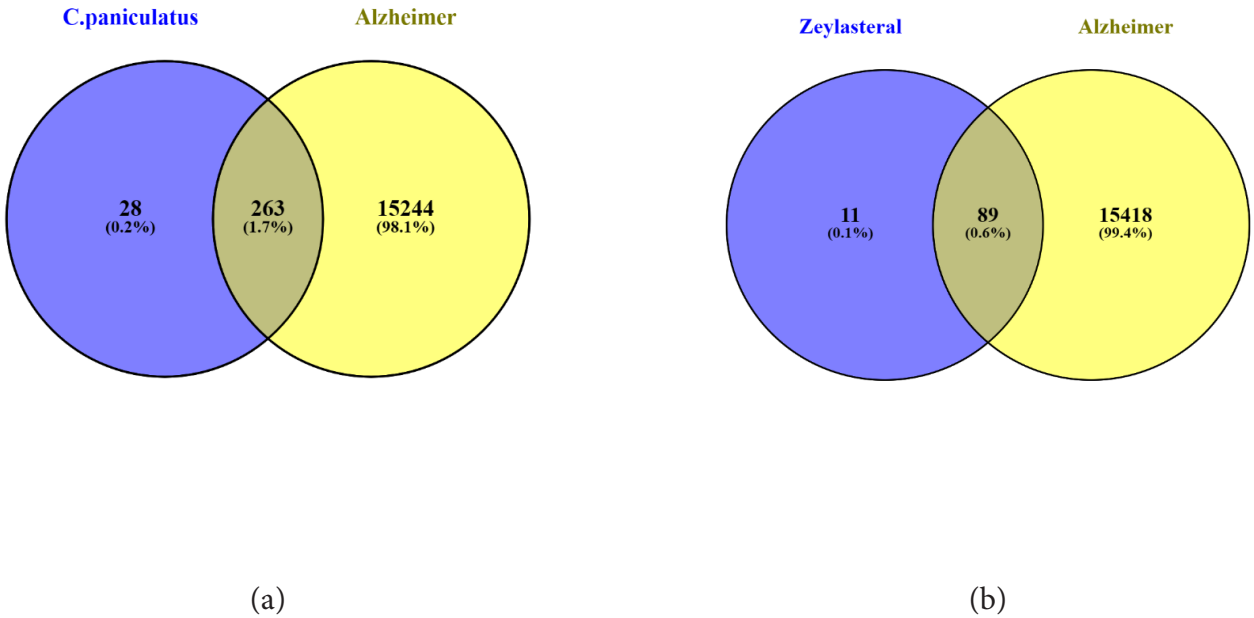


Figure 1: (a) Venn diagram illustrating the common targets between active constituents of *CP* and AD, predicted to be involved in the pathogenesis of AD; (b) common targets between Zeylasteral and AD.

Table 1: List of selected phytochemical constituents of *OS* with their basic information and structure.

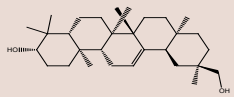
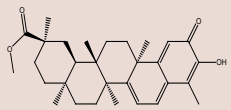
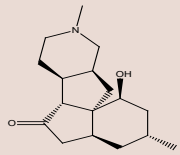
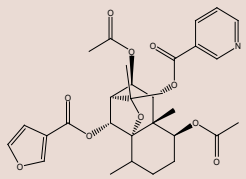
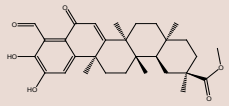
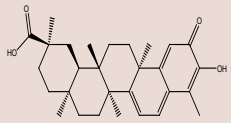
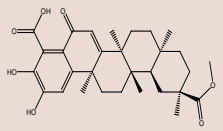
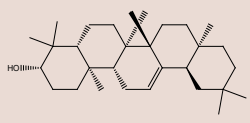
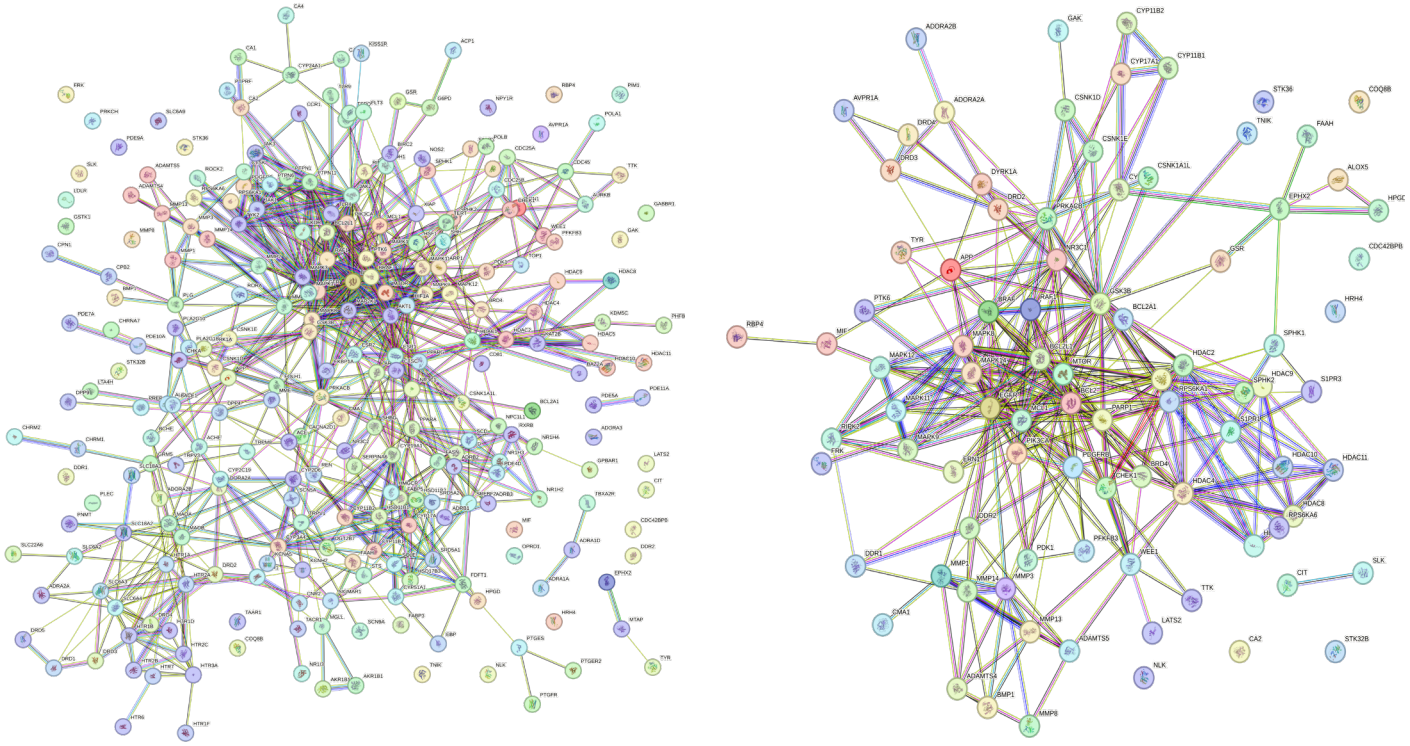
Sl. No.	Name	Molecular Formula	PubChem ID	Canonical smiles	Structure
1.	Paniculatadiol	$C_{30}H_{50}O_2$	101286253	C[C@]12CC[C@@](C[C@H]1C3=CC[C@@H]4[C@]5(CC[C@@H](C([C@@H]5CC[C@]4([C@@]3(CC2)C)C)(C)C)O)C)(C)CO	
2.	Pristimerin	$C_{30}H_{40}O_4$	159516	CC1=C(C(=O)C=C2C1=CC=C3[C@]2(CC[C@@]4([C@@]3(CC[C@@]5([C@H]4C[C@](CC5)(C)C(=O)OC)C)C)C)C)O	
3.	Tetracosanoic acid	$C_{24}H_{48}O_2$	11197	CCCCCCCCCCCCCCCCCCCCCCCC(=O)O	
4.	(1S,3S,8S,9R,12S,14R,16S)-16-hydroxy-5,14-dimethyl-5-azatetracyclo[7.7.0.01,12.03,8]hexadecan-10-one	$C_{17}H_{27}NO_2$	11087072	C[C@@H]1C[C@H]2CC(=O)[C@H]3[C@@]2(C[C@H]4[C@@H]3CCN(C4)C)[C@H](C1)O	
5.	1-Tetracosanol	$C_{24}H_{50}O$	10472	CCCCCCCCCCCCCCCCCCCCCCCCO	
6.	Celapanine	$C_{30}H_{35}NO_{10}$	442518	CC1CC[C@@H]([C@@]2([C@]13[C@@H] ([C@@H]([C@H]([C@@H] 2OC (=O)C4=CN=CC=C4)OC(=O)C)C(O3)(C)C)OC(=O)C5= COC=C5)C) OC (=O)C	
7.	Zeylasteral	$C_{30}H_{38}O_6$	11591321	C[C@]12CC[C@@](C[C@H]1[C@@]3(CC[C@]4(C5=CC(=C(C(=C5C(=O)C=C4[C@]3(CC2)C)C=O)O)O)C)C)(C)C(=O)OC	
8.	Celastrol	$C_{29}H_{38}O_4$	122724	CC1=C(C(=O)C=C2C1=CC=C3[C@]2(CC[C@@]4([C@@]3(CC[C@@]5([C@H]4C[C@](CC5)(C)C(=O)O)C)C)C)C)O	
9.	Zeylasterone	$C_{30}H_{38}O_7$	13945472	C[C@]12CC[C@@](C[C@H]1[C@@]3(CC[C@]4(C5=CC(=C(C(=C5C(=O)C=C4[C@]3(CC2)C)C(=O)O)O)O)C)C)(C)C(=O)OC	
10.	Beta-Amyrin	$C_{30}H_{50}O$	73145	C[C@@]12CC[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]1CC(CC2)(C)C)C	
11.	1-Triacontanol	$C_{30}H_{62}O$	68972	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCO	

Table 2: Docking analysis of zeylasteral with targeted proteins in AD.

Ligand	Protein with PDB ID	Binding affinity	No. of H-bonds	Figure
Zeylasteral	APP (6IYC)	-7.5 kcal/mol	TRP A:653, ILE A:656, ALA A:658	5 (a)
	Raf (3D4Q)	-6.7kcal/mol	TRPB:531, ASNA:512	5 (b)
	JNK (1JNK)	-7.1 kcal/mol	GLN A:75, SER A:72	5 (c)
	mTOR (4JT6)	-6.0 kcal/mol	ARG B:2408, ARG B:2511, ARG B:2505	5 (d)
	PI3K (4L23)	-7.0 kcal/mol	ASN A:920, ARG A:916, ASP A:915	5 (e)
	GSK-3B (5K5N)	-9.5kcal/mol	PTR A:216, ARG A:220, ASP B:200, ASP B:181	5 (f)
	IRE1α (6HX1)	-6.3 kcal/mol	GLY A:721, HIS A:723, CYC A:645	5 (g)
	CKIα (5FQD)	-7.7 kcal/mol	GLU D:151, ASN D:85, ASN D:107	5(h)



(a) (b)

Figure 2: (a) PPI network interaction between CP and AD (b) PPI network interaction between zeylasteral and AD.

Zeylasteral, according to the bioinformatics data, regulates numerous AD-related proteins, including APP, Raf, JNK, mTOR, PI3K, GSK-3 2, IRE1α, and CKI2, which reflect pleiotropic mechanisms of action. Serine/threonine kinase GSK-3β was prioritized among all as it plays a crucial role in tau hyperphosphorylation, Amyloid Precursor Protein (APP) processing, neuroinflammation, and synaptic dysfunction, all of which are important characteristics of AD pathology.[41,42] Hyperactivation of GSK-3β has been consistently associated with the formation of neurofibrillary tangles, aberrant tau aggregation, and neuronal apoptosis, making it a reliable and useful therapeutic target.[43] BDNF and TrkB receptors play a key role in neuronal survival, plasticity, memory, and neurotrophin signaling. The BDNF and TrkB signaling pathway is impaired in Alzheimer's Disease (AD), which suppresses GSK3β and decreases AKT activity. Hence, hyperphosphorylation of tau, neurofibrillary tangle development, and augmented amyloid-β generation are consequences of overactivation of GSK-3β, which eventually leads to synaptic dysfunction and memory loss. Therefore, impaired neurotrophin signaling, a crucial molecular factor in the development of Alzheimer's disease, enables persistent GSK-3β activity.[44-46] GSK-3β is a serine/threonine kinase that is overactive in AD because of the loss of upstream signaling, which results in the tau protein phosphorylation and aggregation into

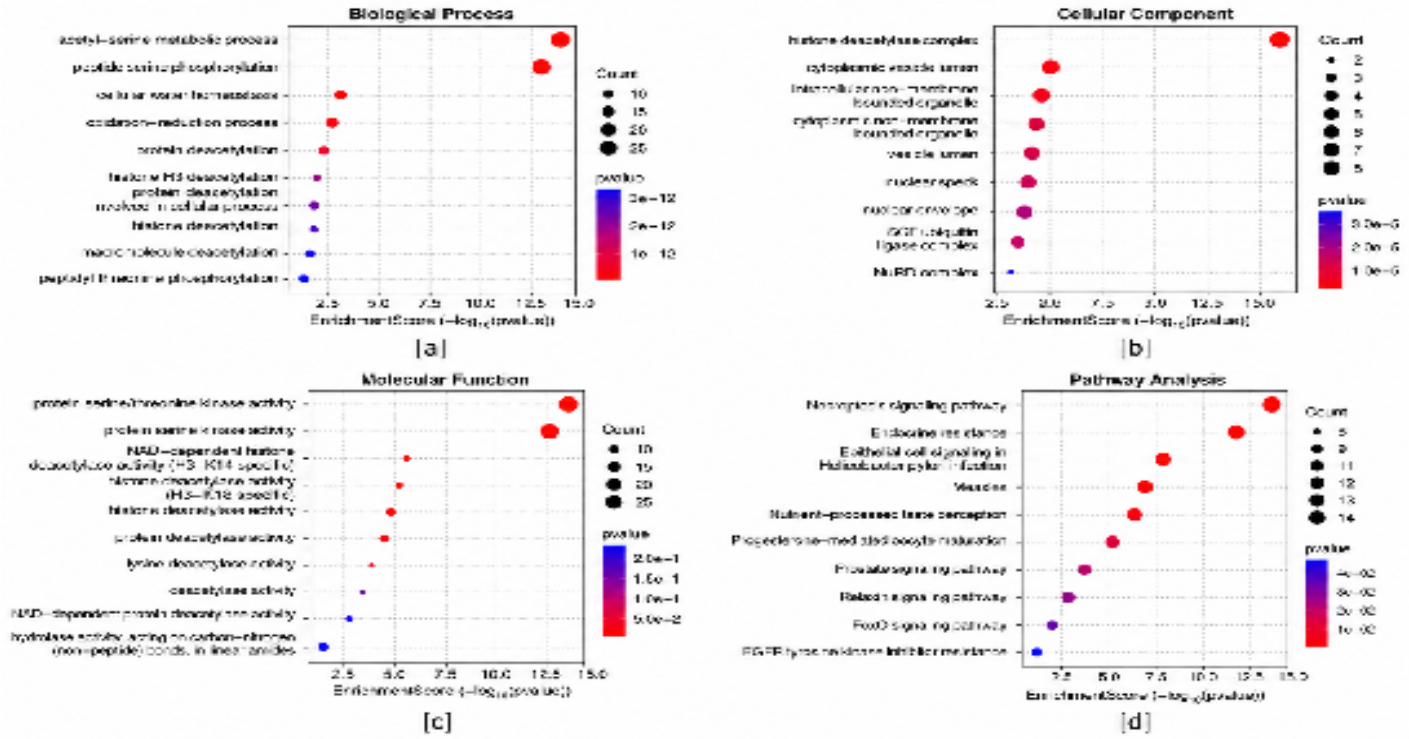


Figure 3: The enrichment analysis chart of top Gene Ontology for categories of (a)Biological Processes (BP), (b)Cellular Components (CC), (c)Molecular Functions (MF), and (d) KEGG pathway analysis.

neurofibrillary tangles. It also alters the processing of Amyloid Precursor Protein (APP), increasing the production of amyloid-β protein as depicted in Figure 6. These outcomes collectively contribute to neuronal death, synaptic dysfunction, and cognitive decline, making GSK3β a key pathological driver and therapeutic target in AD.[47-49] These outcomes indicate that GSK-3β inhibition reduces Aβ pathology and tauopathy. Accordingly, the choice of GSK-3 as the main docking candidate not only indicates the centrality of the latter in AD but also enhances the translational applicability of the results.

Zeylasteral, a quinone, acts on a series of targets associated with AD.[19] Molecular docking was finally used to explore the mechanism of interaction occurring between 8 major targets and zeylasteral. It was found that APP, Raf, JNK, mTOR, PI3K, GSK-3B, IRE1α, and CKIα had strong binding affinity for zeylasteral, as demonstrated by the data. Zeylasteral developed hydrogen-bond interactions with the stated binding-site residues and showed the most favorable projected docking score with GSK-3β (-9.5 kcal/mol) among the targets under investigation. Although docking affinity cannot be equated with experimentally proven enzyme inhibition, these computational results indicate the likelihood of a beneficial zeylasteral-GSK-3β interaction. In order to ascertain whether zeylasteral truly inhibits GSK-3β and to confirm its potency, biochemical GSK-3β kinase assays are necessary, and the current data should be regarded as hypothesis-generating. The possible mechanism underlying AD is GSK-3β activation. GSK-3β was primarily chosen as a therapeutic agent in the treatment of AD.[50]

This study offers high-quality preliminary data supporting the view that quinones should be regarded as a new chemotype in the discovery of AD drugs, combining network pharmacology, enrichment analysis, docking studies, and toxicity analysis. ProTox-3.0 produced positive predictions for respiratory toxicity, immunotoxicity, and nutritional toxicity even though zeylasteral was projected to be inactive for a number of significant organ toxicity and toxicity endpoints. These results raise possible safety issues that need to be taken into account when assessing zeylasteral as a potential treatment for AD. Crucially, ProTox-3.0 forecasts do not determine the prevalence or degree of toxicity in biological systems; instead, they are based on computational models. Therefore, these findings should be considered early warning signs of toxicity that call for more *in vitro* cytotoxicity, immunotoxicity, respiratory-relevant, and suitable *in vivo* safety studies.

In addition, network pharmacology has shown that Zeylasteral has multi-target action, indicating that it may be used in polypharmacological therapy by modulating multiple AD pathways at once. The investigation of quinones as a family of GSK-32 modulators, therefore, creates new opportunities in drug discovery, which can be used to treat the multifactorial character of AD and enhance clinical outcomes where existing treatments do not suffice.

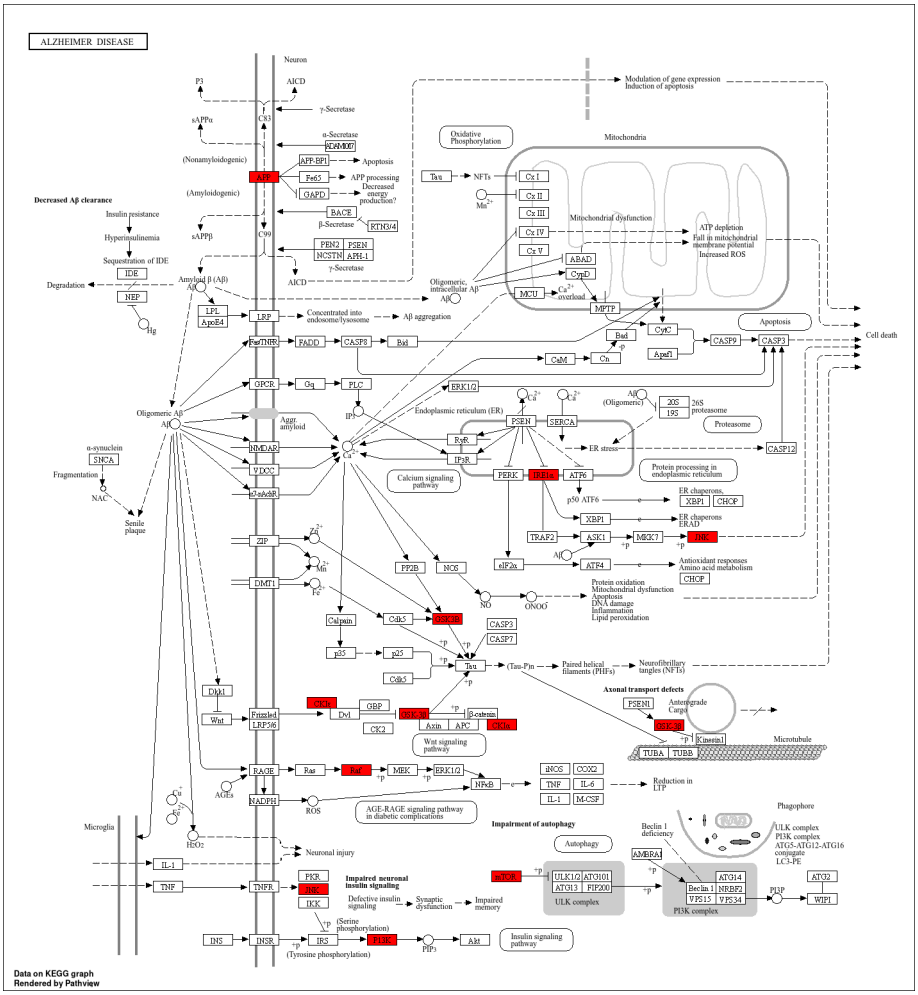


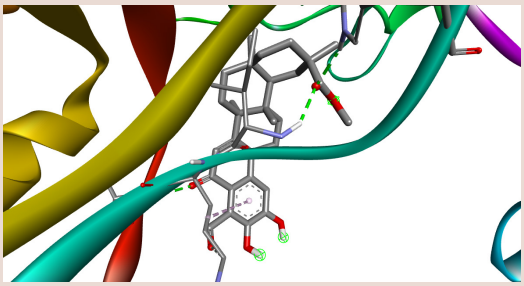
Figure 4: KEGG pathway analysis: Red coloured proteins depict potential targets of Zeylasteral, an active constituent of CP.

Table 3: Toxicity prediction assessment of Zeylasteral.

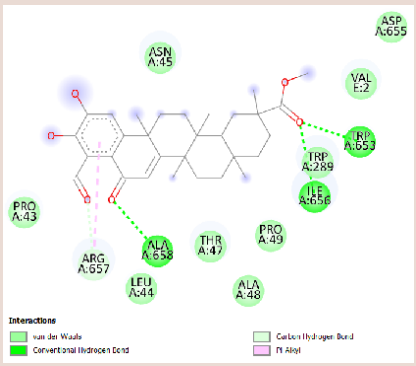
ProTox-3.0 - Prediction of Toxicity of Zeylasteral		
Toxicity	**Target**	**Prediction**
Organ toxicity	Hepatotoxicity	Inactive
Organ toxicity	Neurotoxicity	Inactive
Organ toxicity	Nephrotoxicity	Inactive
Organ toxicity	Respiratory toxicity	Active
Organ toxicity	Cardiotoxicity	Inactive
Toxicity endpoints	Carcinogenicity	Inactive
Toxicity endpoints	Immunotoxicity	Active
Toxicity endpoints	Mutagenicity	Inactive
Toxicity endpoints	Cytotoxicity	Inactive
Toxicity endpoints	Ecotoxicity	Inactive
Toxicity endpoints	Clinical toxicity	Inactive
Toxicity endpoints	Nutritional toxicity	Active
Toxicity endpoints	BBB-barrier	Active

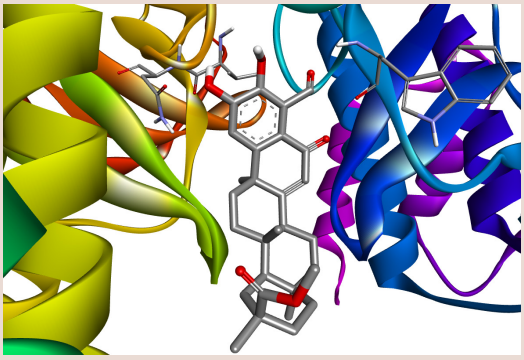
LIMITATIONS

Various limitations like target prediction, network pharmacology, route enrichment, and molecular docking are computational methods, they are unable to prove therapeutic efficacy or direct pharmacological activity. Second, a suitable kinase test must be used to confirm the hypothesized interaction between zeylasteral and GSK-3β biochemically. Further cellular research should ascertain whether zeylasteral affects tau phosphorylation and GSK-3β signaling, and whether these effects are linked to neuronal protection. The evaluation of brain exposure, neuroprotective effects, pathological alterations associated with AD, and cognitive and behavioral outcomes should then be conducted using appropriate *in vivo* AD models. Furthermore, an evaluation of experimental safety is necessary due to the computational toxicity alerts for respiratory, immunotoxic, and nutritional toxicity. Therefore, rather than confirming treatment efficacy, the current data should be viewed as hypothesis-generating and serve as a foundation for further experimental research.

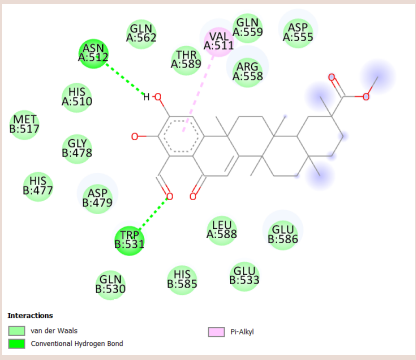


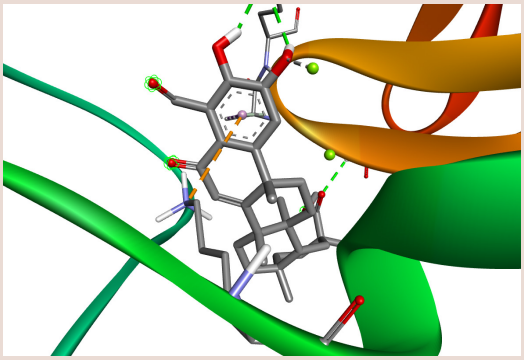
(a)


ASP A:655
ASN A:45
VAL E:2
TRP A:653
TRP A:289
ILE A:656
PRO A:43
ALA A:658
THR A:47
PRO A:49
ARG A:657
LEU A:44
ALA A:48
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Alkyl

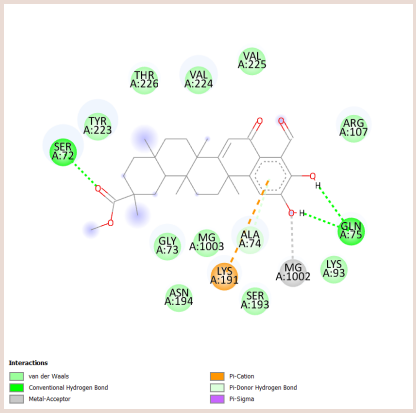


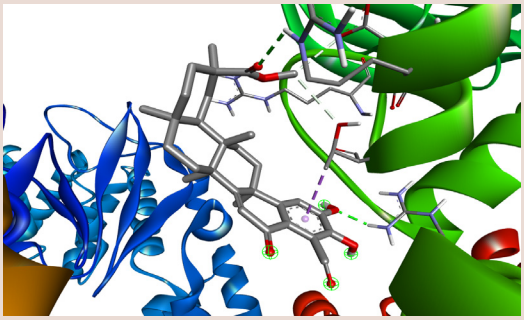
(b)


GLN A:562
GLN A:559
ASP A:555
VAL A:511
ASN A:512
THR A:589
ARG A:558
HIS A:510
MET B:517
GLY B:478
HIS B:477
ASP B:479
LEU A:588
GLU B:586
TRP B:531
GLN B:530
HIS B:585
GLU B:533
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Alkyl

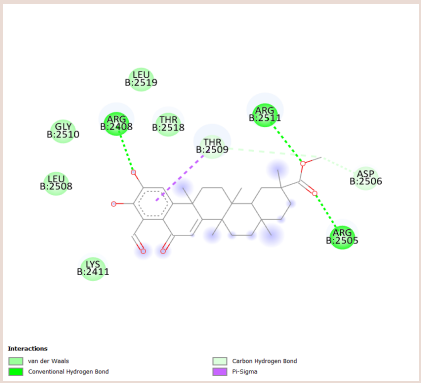


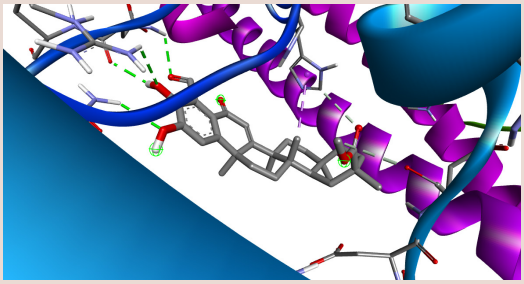
(c)

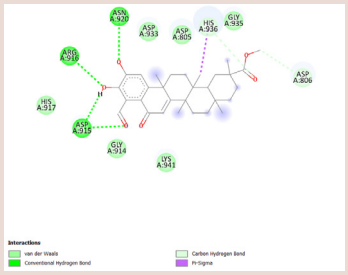

VAL A:225
THR A:226
VAL A:224
TYR A:223
ARG A:107
SER A:72
GLN A:75
GLY A:73
MG A:1003
ALA A:74
LYS A:191
MG A:1002
LYS A:93
ASN A:194
SER A:193
Interactions
van der Waals
Conventional Hydrogen Bond
Metal-Acceptor
Pi-Cation
Pi-Donor Hydrogen Bond
Pi-Sigma



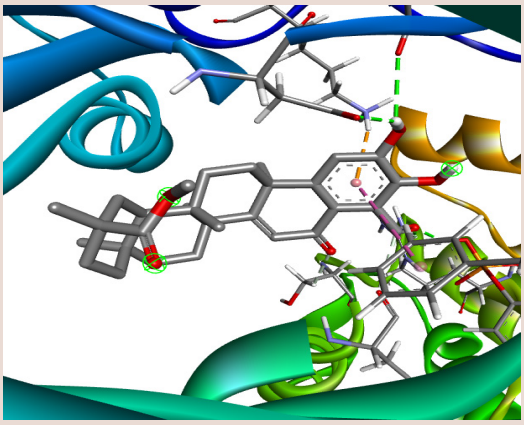
(d)

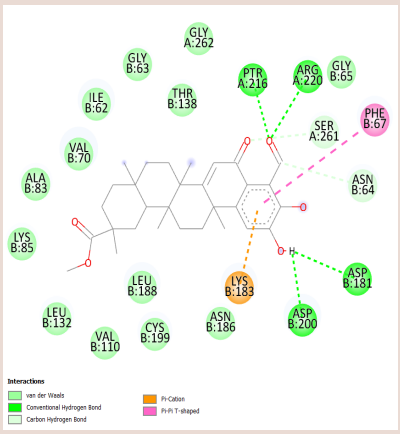

LEU B:2519
GLY B:2510
ARG B:2408
THR B:2518
ARG B:2511
THR B:2509
LEU B:2508
ASP B:2506
ARG B:2505
LYS B:2411
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sigma



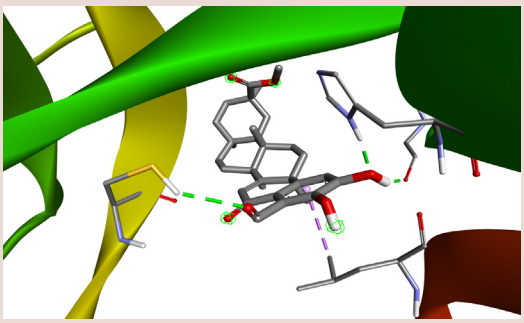


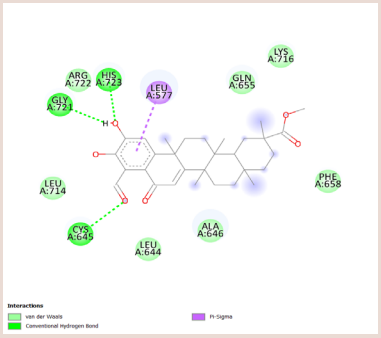
(e)



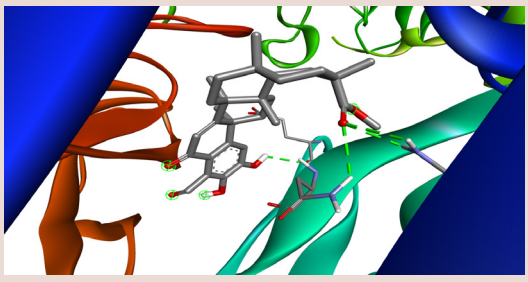


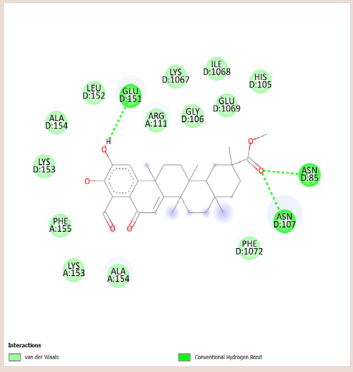
(f)





(g)





(h)

Figure 5: Docking of Zeylasteral within the active site of target proteins, showing key interactions relevant to Alzheimer's disease.

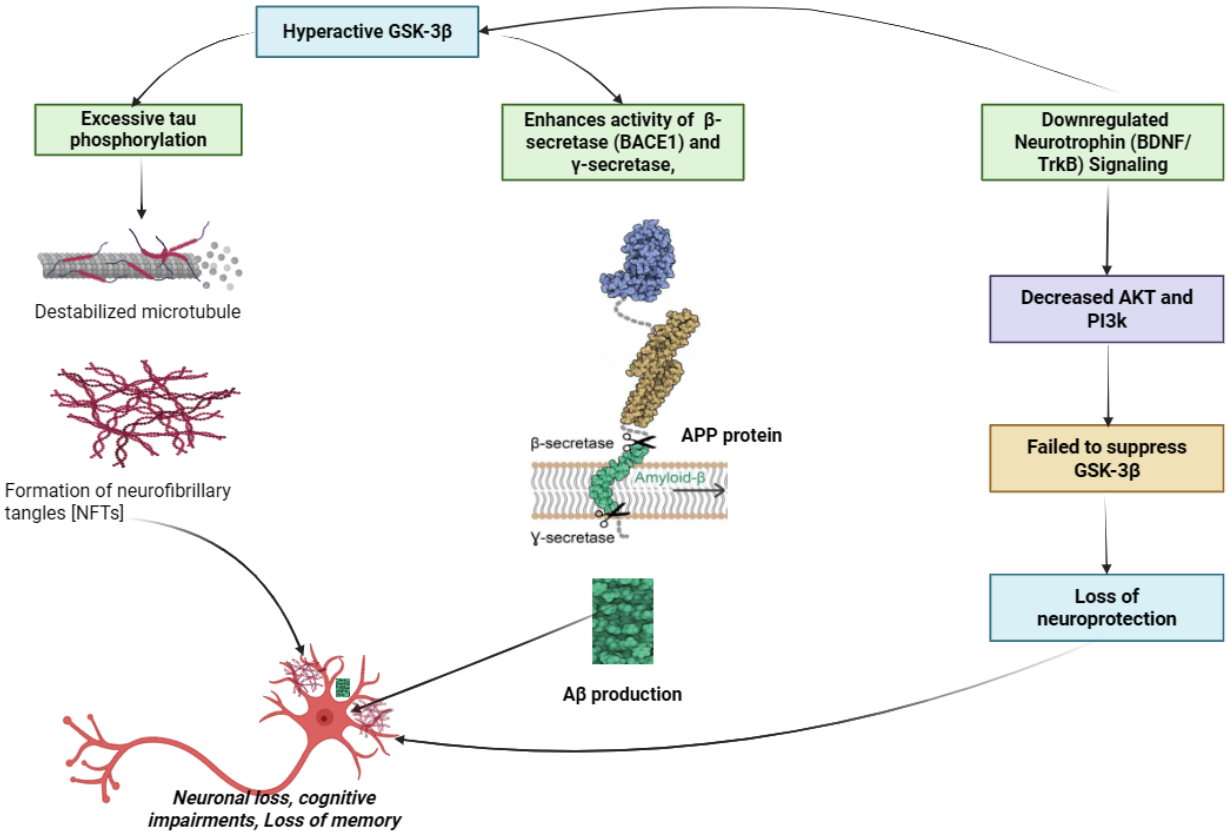


Figure 6: Role of GSK-3β involved in various pathways influencing AD pathogenesis.

CONCLUSION

This research presents an innovative approach that elucidates the therapeutic mechanisms of one active constituent of CP, which acts on multiple targets in AD. Using molecular docking and network pharmacology, key target proteins for AD treatment were identified. In conclusion, the present integrated network pharmacology and molecular docking analysis identified zeylasteral as a computationally prioritized phytoconstituent of C. paniculatus with multiple predicted interactions with Alzheimer's disease-associated targets. Among the investigated proteins, GSK-3β showed the most favorable predicted docking interaction with zeylasteral. These findings provide a mechanistic hypothesis regarding the possible involvement of GSK-3β and related signaling pathways; however, they do not establish direct enzyme inhibition or therapeutic efficacy against Alzheimer's disease. Further experimental validation is therefore essential. *In vitro* studies should initially investigate direct GSK-3β inhibition, effects on tau phosphorylation, neuronal viability, oxidative/ neuroinflammatory responses, and other AD-relevant molecular endpoints. Subsequently, appropriate *in vivo* Alzheimer's disease models should be used to evaluate pharmacokinetics, brain exposure, neuroprotective effects, neuropathological changes, and cognitive and behavioral outcomes.Prior to additional therapeutic development, the anticipated respiratory, immunotoxicity, and nutritional toxicity alarms should also be studied experimentally. Therefore, rather than being a proven anti-Alzheimer's medication, zeylasteral should currently be regarded as an interesting computational lead for additional research.

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ABBREVIATIONS

AD: Alzheimer's disease; **Aβ:** Amyloid-beta; **APP:** Amyloid precursor protein; **BDNF:** Brain-Derived Neurotrophic Factor; **BP:** Biological Processes; **CC:** Cellular Components; **CKIα:** Casein kinase I alpha; **CP:** Celastrus paniculatus; **FDA:** Food and Drug Administration; **GO:** Gene Ontology; GSK-3β**:** Glycogen Synthase Kinase-3 beta; **IRE1α:** Inositol-requiring enzyme 1 alpha; **JNK:** c-Jun N-terminal kinase; **KEGG:** Kyoto Encyclopedia of Genes and Genomes; **MF:** Molecular functions; **mTOR:** Mechanistic target of rapamycin; **NFTs:** Neurofibrillary tangles; **NMDA:** N-methyl-D-aspartate; **PDB:** Protein Data Bank; **PDBQT:** Protein Data Bank, Partial Charge (Q), and Atom Type (T) format; **PI3K:** Phosphoinositide 3-kinase; **PPI:** Protein-Protein Interaction; **SDF:** Structure Data File; **STRING:**

Search Tool for the Retrieval of Interacting Genes/Proteins; **WHO:** World Health Organization.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Bharat Mishra: Conceptualization, Supervision, writing the original draft, and editing the final version of the manuscript. **Archita Tiwari:** Methodology, literature collection, Data Curation, and writing the original version of the manuscript. **Bhupendra Singh:** Software; Network Pharmacology and Docking study performed; **Shivendra Mani Tripathi:** Data Curation and Formal Analysis; **Sudhanshu Mishra:** Final Review, Editing, and Writing of the manuscript.

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