

# ***In silico* Analysis of Selected Phytocompounds of *Cocculus hirsutus* as Potent Inhibitors of Tau Aggregation in Alzheimer's Disease**

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**Abstract** In the current investigation, the selected phytocompounds of *Cocculus hirsutus* were screened for potent tau aggregation inhibitors for Alzheimer's disease through *in silico* molecular docking. Approximately 19 phytocompounds from *Cocculus hirsutus* were selected and assessed for their drug likeness based on *rule-of-five* using Swiss ADME. The phytocompounds that passed *rule-of-five* were subjected to *in silico* analysis through molecular docking on Cyclin-dependent kinase 5 (CDK5) and Glycogen synthase kinase 3 beta (GSK3B) using Molegro Virtual Docker v6.0. Molecular docking studies. The molecular interaction on CDK5 and GSK3B enzymes indicate their role in the tau protein aggregation inhibitory activity of the phytocompounds. *In silico* analysis showed that hirsutine and cohirsinine bind effectively at the active site of target proteins with a MolDock score of -115.08 and -107.29 respectively. The MolDock scores, ReRank scores and interaction poses of the phytocompounds were compared with the known inhibitors of CDK5 and GSK3B enzymes. The present research created a new perspective in understanding hirsutine and cohirsinine phytocompounds as effective inhibitors of tau aggregation and further research *in vitro* and *in vivo* may confirm their therapeutic potential in Alzheimer's.

**Keywords** *Cocculus hirsutus*, Tau Aggregation, CDK5, GSK2B, Molecular Docking, *In silico*

## **1. Introduction**

Alzheimer's disease (AD), a chronic irreversible neuro-disorder in which the progressive degeneration of brain neuronal cells causes gradual decline in cognitive skills [1]. About 50 million people around the globe have some form of dementia and in India, it's almost 4 million. It was predicted that the statistics may reach 152 million globally by 2050 (Alzheimer's disease facts and figures, 2019). Neurodegeneration is mainly triggered by abnormal phosphorylation and self-polymerization of tubulin associated tau protein, which leads to microtubular disorganization and intracellular aggregation of polymerized helical tau filaments with straight filaments (tau-tau interaction) to form neuritic plaques, neuropil threads and neurofibrillary tangles [2]. The accumulation of fibrillary tangles in the neurons along with hyperphosphorylated tau proteins causes disturbances in microtubule network, affects the axonal communication

and leads to neuronal dysfunction [3]. Protein kinases such as CDK5 and GSK3B played a vital role in pathological hyperphosphorylation of microtubule associated protein tau in brain. The findings suggest that CDK5 and GSK3B could be the potent targets in regulation of abnormal phosphorylation of tau in Alzheimer's disease [4]. Considering the multiple failures of clinical trials targeting various pathways in Alzheimer's, drives interest in finding potent inhibitors of tau aggregation from natural sources [5]. In search of natural phytochemical with potent tau aggregation inhibitor activity, the research focuses on selected phytochemicals from *Cocculus hirsutus*.

*Cocculus*, an ancient ayurvedic genus with some 157 species of angiosperms, is distributed throughout the tropical lands. Literature suggests that *Cocculus hirsutus* have good traditional and therapeutic value due to presence of phytochemicals such as alkaloids, flavonoids, triterpenes, volatile compounds, etc. [6] These bio-active phytochemicals are responsible for diversified pharmacological properties such as detoxifier, analgesic, anti-diarrheal, diuretic, antirheumatic, hypotensive, spasmolytic, anti-inflammatory, etc. [7] The bioactive phytochemicals of *Cocculus hirsutus* have been less explored to possess neuroprotective potentials for the treatment of AD.

*In silico* docking research can be useful to predict the binding affinity of a phytochemical and the target protein and play an important role in finding an inhibitor via structure-based drug design [8]. In this investigation, CDK5 and GSK3B enzymes have been analyzed for screening of potent tau aggregation inhibitors from *Cocculus hirsutus* through *in silico* molecular docking with Molegro Virtual Docker v6.0.

## 2. Materials and Methods

### 2.1. Drug-Like Parameters

Drug-like parameters such as molecular weight (MW), lipophilicity ( $C \text{ Log } P_{o/w}$ ), the number of hydrogen bond donors (nON) and the number of hydrogen bond acceptors (nOHNH) were calculated for all the selected ligands by using a free web tool SwissADME, Swiss Institute of Bioinformatics and evaluated for the violations of *rule-of-five* [9].

### 2.2. Retrieval and Processing of Selected Target Proteins

Three-dimensional X-ray crystallographic structures of cyclin-dependent kinase 5 complexed with roscovitine (PDB ID: 1UNL with resolution of 2.2 Å) [10] and glycogen synthase kinase 3 beta complexed with indirubin-3'-monoxime (PDB ID: 1Q41 with resolution of 2.1 Å) [11] were retrieved from Protein Data Bank (RCSB PDB: Homepage). The raw proteins were treated by

removing ligands and water molecules, adding implicit hydrogen atoms to adjust valency and assigning bond angles, bond ordering and topologies to correct crystallographic abnormalities. Then the proteins were subject to refinement and energy minimization by using the MM2 force field before performing docking studies.

### 2.3. Validation of Selected Target-Ligand Complex

For the selected target proteins, the current Molegro Virtual Docker v6.0 docking software had to be validated to ensure that ligands docked with this software reflect precise binding interactions with the target receptor. In view of this, CDK5 (PDB ID: 1UNL) and GSK3B (PDB ID: 1Q41) were validated with their co-crystallized inhibitors roscovitine and indirubin-3'-monoxime respectively.

### 2.4. Retrieval and Processing of Selected Ligands

The 3D structures of all the Lipinski rule passed ligands from *Cocculus hirsutus* were retrieved from PubChem online database, National Library of Medicine, USA. Using ChemBioOffice Ultra 14.0 suite, ligands were preprocessed by adding explicit hydrogens, neutralizing charged groups and minimizing the energy. The processed ligands were optimized and converted into mol file.

### 2.5. Molecular Docking Studies

Molecular docking studies were performed to understand the ligand-receptor interaction and affinity of selected phytochemicals against CDK5 and GSK3B enzymes using Molegro Virtual Docker v6.0 in personal computer with 12GB RAM and an Intel 10<sup>th</sup> generation, Core i5-10210U CPU @1.60 gigahertz processor running Windows 10. Initially, docking protocol was validated and perform redocking stimulations to recover the crystallographic position of the ligand. With default input parameters, all phytochemicals were docked to the specified active site in the target protein. The MolDock scores (GRID), ReRank scores and HBond of the ligands docked into the target proteins were calculated, best interaction poses were evaluated and compared with the co-crystallized inhibitors of CDK5 and GSK3B enzymes to find the best hit of all the phytochemicals.

## 3. Results and Discussions

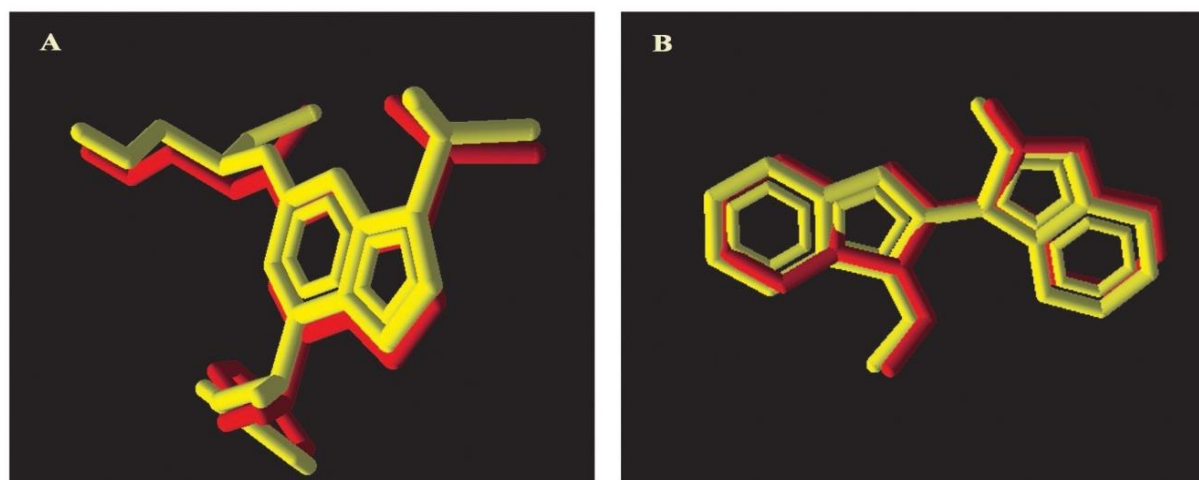
### 3.1. Analysis of Drug-Like Parameters

The independent descriptors were predicted for each of the 19 phytochemicals from *Cocculus hirsutus*. The phytochemicals that passed the Lipinski's rule of five ( $MW < 500 \text{ Da}$ ;  $C \text{ Log } P_{o/w} < 5$ ;  $n\text{OHNH} < 10$  and  $n\text{ON} < 5$ ) with the drug-likeness scores were shown in Table 1.

**Table 1.** Drug-likeness parameters of the selected ligands

Name of the phytochemical	MW	C Log $P_{o/w}$	nON	nOHNH
Coclaurine	285.34	2.35	4	3
Cohirsine	329.39	2.38	4	0
Cohirsinine	315.36	2.05	4	1
Cohirsitine	399.44	2.33	7	0
Cohistinine	301.38	2.33	4	1
Corsutine	405.44	0.96	8	2
Haiderine	315.36	1.6	5	2
Hirsutine	368.47	3.22	4	1
Jamtine N-oxide	360.42	1.56	5	1
Jamtinine	357.40	2.15	5	0
Liquiritin	418.39	0.40	9	5
Magnoflorine	342.41	1.87	4	2
Quercetin	302.24	1.23	7	5
Shaheenine	301.34	1.66	4	2

MW: Molecular weight, C Log  $P_{o/w}$ : Consensus octanol-water partition coefficient, nON: number of H-bond acceptors, nOHNH: number of H-bond donors



**Figure 1.** The interaction poses of the co-crystallized (internal) ligand (Yellow) and extracted internal ligand (Red). **A)** Roscovitine **B)** Indirubin-3'-monoxime

### 3.2. Target-Ligand Complex Validation

The molecular docking interactions and the interaction poses of the co-crystallized (internal) ligand and extracted internal ligand of the docked target protein-ligand complex structures served as control docking models, as shown in Figure 1.

### 3.3. Molecular Docking Analysis

The pharmacological activity of selected ligands from *Cocculus hirsutus* against cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase 3 beta (GSK3B) target proteins was analyzed using *in silico* molecular docking studies. The docking results indicated that out of all docked phytochemicals hirsutine and cohirsinine

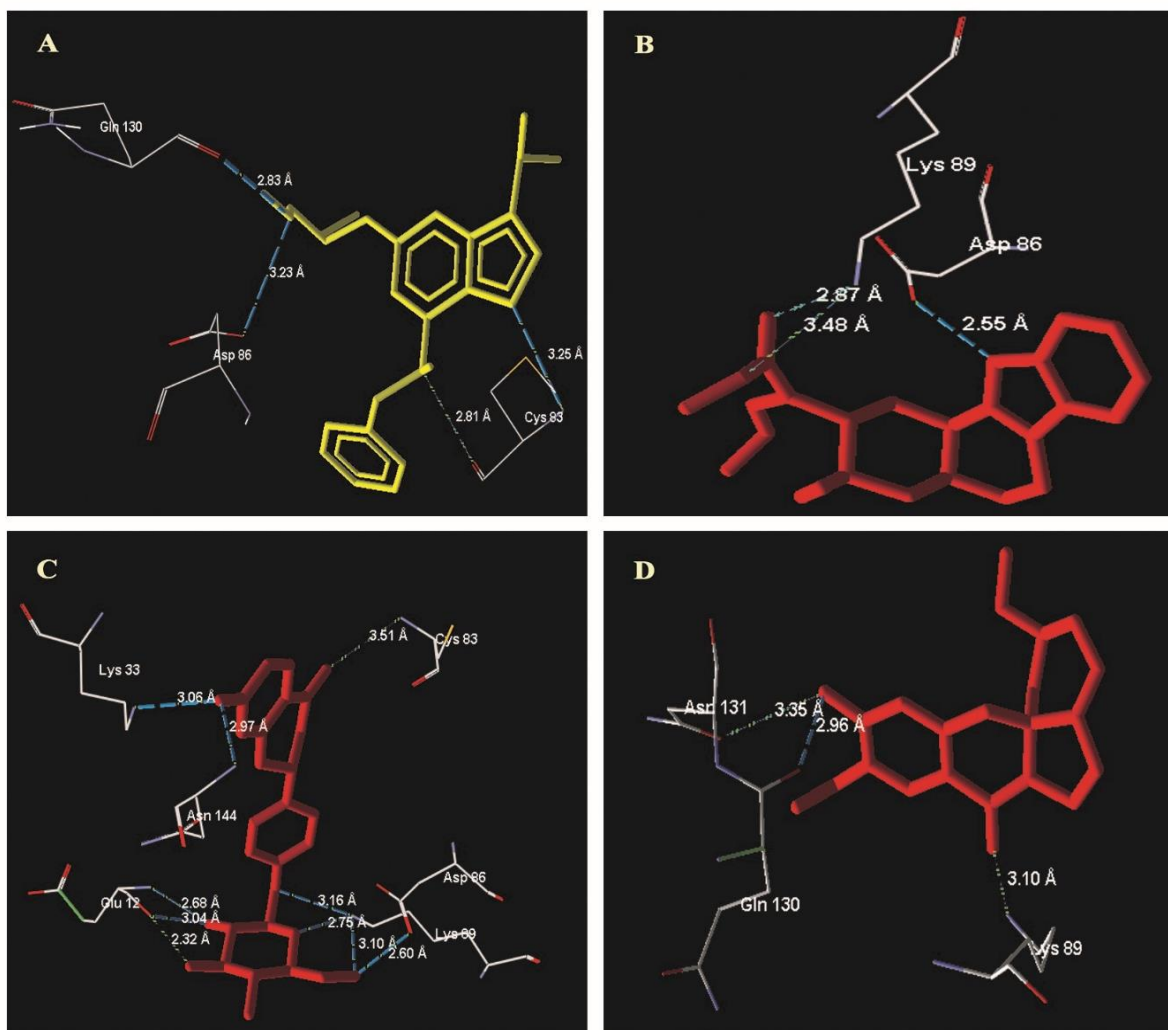
shows highest binding affinities towards the active sites of CDK5 and GSK3B respectively. The MolDock, ReRank and HBond scores of the top interacting phytochemicals were calculated. The best interaction poses were evaluated and compared with the known inhibitors of CDK5 and GSK3B enzymes (Table 2).

The significant inhibitory effects of certain phytochemicals on hyperphosphorylation of tau protein in AD may be due to their strong binding affinities.

Hirsutine, an indole alkaloid of *Cocculus hirsutus* pharmacologically, is used as anti-metastatic [12], cardioprotective [13], anti-arrhythmic [14], anti-inflammatory and ion channel blocker [15]. The research findings may support neuroprotective potentials of hirsutine against Alzheimer's disease [16]. However, whether hirsutine can be bound to cyclin-dependent

kinase 5 is not yet studied. Therefore, we use molecular docking analysis to evaluate all the selected phytochemicals and to analyze the probability of hirsutine binding to the CDK5 target. The docking interactions and bond lengths of top interacting ligands were stated (Table 3) and the results indicated that hirsutine

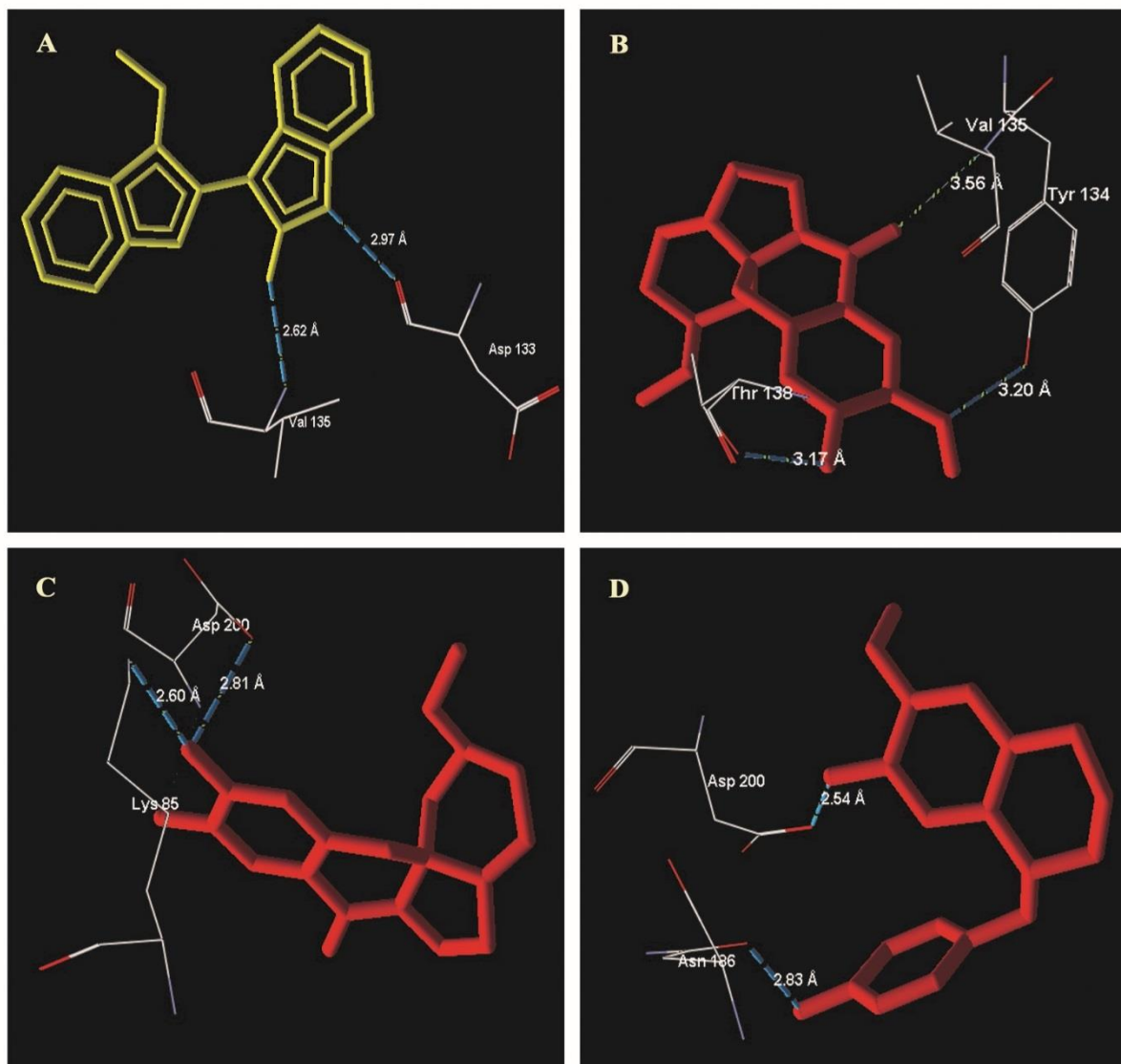
may have good binding affinity to the CDK5 target protein at several sites such as Asp86 and Lys89 with a MolDock Score of -115.08 kcal/mol (Figure 2B). Hirsutine exhibited appreciable binding interactions when compared with the binding interactions of the known inhibitor roscovitine (Figure 2).



**Figure 2.** Molecular docking interactions with CDK5 (1UNL). A) Roscovitine B) Hirsutine C) Liquiritin D) Cohirsinine

**Table 2.** MolDock score, ReRank and HBond scores of top 3 interacting bioactive phytochemicals

Target protein	Ligand	MolDock score	ReRank score	HBond
CDK5 (1UNL)	Roscovitine	-137.77	-63.59	-6.91
	Hirsutine	-115.08	-82.21	-5.60
	Liquiritin	-102.59	-87.21	-18.83
	Cohirsinine	-96.23	-66.65	-8.01
GSK3B (1Q41)	Indirubin-3-monoxime	-122.87	-99.16	-7.22
	Cohirsinine	-107.29	-88.47	-4.32
	Shaheenine	-95.45	-64.95	-5.03
	Coclaurine	-87.10	-73.17	-7.50



**Figure 3.** Molecular docking interactions with GSK3B (1Q41). **A)** Indirubin-3-monoxime **B)** Cohirsinine **C)** Shaheenine **D)** Coclaurine

Cohirsinine, an isoquinolone alkaloid of *Cocculus hirsutus*, has been reported with less or no significant pharmacological properties [17]. However, the aqueous extracts of *Cocculus hirsutus* containing alkaloidal constituents exhibited substantial diuretic, antidiabetic and laxative effects [18]. It is still not known whether cohirsinine could inhibit GSK3B target protein. Therefore, we use molecular studies to assess all the selected ligands and to analyze the possibility of cohirsinine binding to the

GSK3B target protein. The docking interactions and bond lengths of top interacting ligands were reported (Table 4) and the results indicated that cohirsinine may have good binding affinity to the GSK3B target protein at several sites such as Thr138, Tyr134 and Val135 with a MolDock Score of -107.29 kcal/mol (Figure 3B). Cohirsinine exhibited significant binding interactions when compared with the known inhibitor Indirubin-3-monoxime (Figure 3).

**Table 3.** Hydrogen bonding interactions between ligands and CDK5 target protein.

Top interacting ligands		Target Protein (CDK5)			Bond length (Å)
Ligand	Ligand atom	AA Residues	PDB atom ID	PDB atom name	
Roscovitine	N (Enamine)	Cys83	676	O	2.81
	O (Alcohol)	Gln130	1058	O	2.83
	O (Alcohol)	Asp86	702	O	3.23
	N (Imine)	Cys83	673	N	3.25
Hirsutine	N (Pyrrole)	Asp86	702	OD1	2.55
	O (Carbonyl)	Lys89	729	NZ	2.87
	O (Carboxyl)	Lys89	729	NZ	3.48
Liquiritin	O (Alcohol)	Glu12	98	O	2.32
	O (Alcohol)	Asp86	703	OD2	2.60
	O (Alcohol)	Glu12	95	N	2.68
	O (Ether)	Lys89	729	NZ	2.75
	O (Enol)	Asn144	1166	N	2.97
	O (Alcohol)	Glu12	98	O	3.04
	O (Enol)	Lys33	269	NZ	3.06
	O (Alcohol)	Lys89	729	NZ	3.10
	O (Enol)	Lys89	729	NZ	3.16
	O (Carbonyl)	Cys83	673	N	3.51
Cohirsinine	O (Enol)	Gln130	1058	O	2.96
	O (Carbonyl)	Lys89	729	NZ	3.10
	O (Enol)	Asn131	1070	OD1	3.35

**Table 4.** Hydrogen bonding interactions between ligands and GSK3B target protein.

Top interacting ligands		Target Protein (CDK5)			Bond length (Å)
Ligand	Ligand atom	AA Residues	PDB atom ID	PDB atom name	
Indirubin-3-monoxime	O (Enol)	Val135	746	N	2.62
	N (Pyrrole)	Asp133	729	O	2.97
Cohirsinine	O (Enol)	Thr138	774	OG1	3.17
	O (Enol)	Tyr134	745	OH	3.20
	O (Carbonyl)	Val135	746	N	3.56
Shaheenine	O (Enol)	Lys85	372	NZ	2.60
	O (Enol)	Asp200	1293	OD2	2.81
Coclaurine	O (Enol)	Asp200	1293	OD2	2.54
	O (Enol)	Asn186	1187	OD1	2.83

In this research, we reject those phytochemicals that violate *rule-of-five*, although they may have efficient binding interactions with the amino acids in the target proteins. All the selected phytochemicals of *Cocculus hirsutus* were docked with a minimum of 25 runs against

cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase 3 beta (GSK3B) target proteins. One best pose from each phytochemical was selected and compared with the hydrophobic interactions of validated inhibitors. The higher negative value indicates the strong binding

interactions between the ligand and target protein. However, we have found that alkaloidal phytocompounds of *Cocculus hirsutus* such as hirsutine and cohirsinine have substantial binding affinity towards CDK5 and GSK3B respectively.

#### 4. Conclusion

In conclusion, *in silico* analysis proposed that hirsutine and cohirsinine bioactive alkaloids have potential to interact with cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase 3 beta (GSK3B) target proteins respectively and these findings will be helpful to researchers for their future investigation to confirm their therapeutic potentials in Alzheimer's disease.

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