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Identification Of Potential Natural Inhibitors Against Human Acetylcholinesterase Enzyme From Tubers Of Pueraria Tuberosa (Willd.): An Insilico Investigation

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Abstract
Human acetylcholinesterase enzyme acts as a key regulator of cholinergic signaling and its presence in post-synaptic membrane results in neuronal signal transmission termination via hydrolysis of neurotransmitter acetylcholine into acetate and choline. Acetylcholinesterase(AchE) inhibitors have proven therapeutic effect in neurodegenerative diseases like Alzheimer's disease (AD) where AchE inhibitors could temporally compensate the cholinergic deficit by preventing acetylcholine hydrolysis and improving cholinergic transmission ultimately resulting in improved cognitive behaviour of AD patients. The present study aims to identify potential natural acetylcholinesterase(AchE) inhibitors from tubers of *Pueraria tuberosa*. Three-dimensional structure of human acetylcholinesterase enzyme (PDB ID: 7E3H) and 15 phytochemicals isolated from tubers of *Pueraria tuberosa* were downloaded from PDB and PubChem database, respectively. The pharmacological properties of phytochemicals were evaluated by the SwissADME tool to determine their Absorption, Distribution, Metabolism, and Excretion and Toxicity properties. The molecular docking interactions between acetylcholinesterase enzyme and phytochemicals were done using AutoDock Vina. Visualization of the docking complex was done in the Discovery Studio package. All phytochemicals exhibited strong binding with the Acetylcholinesterase as revealed by molecular docking analysis. Three compounds i.e, Deoxypodophyllotoxin, Tuberosin and cis-4-Decenedioic acid with a binding energy of -9.745,-9.733, and -6.8 kcal mol-1 are best suited candidate for devolvement as an Acetylcholinesterase inhibitor due to their high binding energies , Drug likeliness and favorable pharmacokinetics properties.

Keywords: Acetylcholinesterase, Molecular Docking, *Pueraria tuberosa*, Deoxypodophyllotoxin, Tuberosin, cis-4-Decenedioic acid

Introduction

Human acetylcholinesterase enzyme acts as a key regulator of cholinergic signaling and its presence in post-synaptic membrane results in neuronal signal transmission termination via hydrolysis of neurotransmitter acetylcholine into acetate and choline (Singh & Gupta, 2017). Acetylcholinesterase(AchE) inhibitors have proven therapeutic effect in neurodegenerative diseases like Alzheimer's disease (AD) where AchE inhibitors could temporally compensate the cholinergic deficit by preventing acetylcholine hydrolysis and improving cholinergic transmission ultimately resulting in improved cognitive behaviour of AD patients. The importance of AchE inhibitors can be gauged by the fact that out of five commercially available treatment in AD four i.e, donepezil, galantamine , rivastigmine and tacrine are AchE inhibitors(Tang et al., 2019). Natural products have been a valuable source of developing medicines for centuries with a large number of phytochemicals such as Isorhamnetin(Jamali-Raeufy et al., 2019), Apigenin(Cavallaro et al., 2018) Linalool(Xu et al., 2017), identified as potent AchE inhibitors. The therapeutic potential of phytochemicals of *Pueraria tuberosa* such Daidzin, Genistin, Mangiferin, Puerarin, and Tuberosin. as have been earlier reported as Beta-site amyloid precursor protein cleaving enzyme-1(BACE-1) inhibitors and improved in AD modelled Drosophila flies (Ahuja et al., 2021). The current study aims at identifying natural acetylcholinesterase inhibitors from phytochemical isolated from *Pueraria tuberosa* using insilico approaches like Molecular docking for potential treatment in AD.

2. Material and methods

Molecular Docking

The crystal structure of human acetylcholinesterase in complex with inhibitor donepezil with a resolution of 2.45 Å was already well established (Dileep et al., 2022). The three dimensional structure of acetylcholinesterase enzyme having PDB ID: 7E3H was retrieved in PDB Format from the Research Collaboratory for structural Bioinformatics(RCSB)-Protein

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Data Bank. The 3D structure of acetylcholinesterase enzyme was prepared for docking by removing nonspecific molecules and water in UCSF Chimera. Natural compounds(15) isolated from tubers of *Pueraria tuberosa* were screened for identifying any potential inhibitors of acetylcholinesterase enzyme. The details of natural compound screened in the present study are mentioned in Table 1. Chemical structure of these 15 compounds were retrieved in SDF format from PubChem database. Generation of PDB structure of these compounds was done using Open Babel chemical toolbox(Ahuja et al., 2021). Energy minimization of all 15 ligands (natural compounds) was done in UCSF Chimera. Molecular Docking of natural compounds with acetylcholinesterase enzyme was performed using Autodock vina. The center point coordinates for grid box set was X= -44.0107, Y= 35.5543, and Z= 35.5543. While the size dimensions of Grid box were X=23.8713 , Y=18.7294 and Z= 18.0136.

Drug Likeness

With significant progress in computer aided drug design the calculations of Absorption, Distribution, Metabolism, and Excretion (ADME) properties of a therapeutic compound in the discovery phase can significantly lower the chances of pharmacokinetics-related failures in subsequent clinical stages. Drug likeness and pharmacokinetic properties of 15 compounds isolated from tubers of *Pueraria tuberosa* were predicted using SWISSADME.

Results and Discussion

The utility of molecular docking approach in identification of new drug targets has significant prominence in ligand based computer aided drug discovery(Ahuja et al., 2021). Nowadays, a large amount of data can be quickly analysed and annotated from drug libraries significantly reducing the cost and time of drug discovery (Ahuja et al., 2021). The molecular docking results revealed that all 15 phytochemicals showed strong binding affinities with human acetylcholinesterase enzyme with l-Threitol(-4.64 kcal mol-1) exhibiting lowest binding energy while Dihydroergocornine(12.863 kcal mol-1) exhibiting highest binding energy Table 1. The presence of unfavorable acceptor-acceptor bond at Asp74 residue may account for the lowest binding energy despite the presence of 3 hydrogen bonds at Tyr124,Ser125 and Asn87 residue(Table 1). Several studies have highlighted the critical role of Hydrogen bonds in protein ligand complexes stabilization(Ahuja et al., 2021; Sakkiah et al., 2012). In case of Dihydroergocornine with highest binding energy the docking interactions revealed several interaction such as hydrogen bonds(Tyr124, Ser125), carbon hydrogen bonds(Gly121,Gly120,Thy133), Pi Cation (Tyr341),Pi-Pi stacked (Trp86), Pi-Alkyl(Trp286). Despite the high binding energy of Dihydroergocornine it may not be the best candidate for acetylcholinesterase inhibition due to its pharmacokinetic properties such as low Blood brain barrier (BBB) permeability (Table 3) and it also does not qualify the criteria for Lipinski Rule of five for accessing the drug likeliness(Table 2). Lipinski's rule of five for drug likeliness states that for any molecules to be considered a potential drug candidate it should qualify five criteria : number of H-bond acceptors ≤10, number of H-bond donors ≤5, molar refractivity from 40 to 130, molecular weight ≤ 500, and LogP ≤5(Lipinski et al., 2001). Out of 15 compounds analysed in the present study only three compounds i.e, Deoxypodophyllotoxin, Tuberosin cis-4-Decenedioic acid having a binding energy of 9.745, -9.733,- 6.8 kcal mol-1 respectively qualify for all rule of drug likelikeness i.e, Lipinski, Veber, Ghose, Egan and Muegge rule(Table 1 and Table 2) . These 3 compounds also qualify important pharmacokinetic properties i.e, High GI absorption and BBB permeability. The docking interaction of Deoxypodophyllotoxin showed several interaction such as carbon hydrogen bonds(His447, Ser125, Gly120,Asn87,Thr83), Pi-Donor hydrogen bonds(Tyr124) Pi-Pi stacked (Tyr337), Pi-Pi T shaped (Phe338), Alkyl(Leu130) Pi-Alkyl(Trp86).Similarly Tuberosin docking interaction revealed conventional hydrogen bond at (Tyr124), Pi- Donor hydrogen bond (Phe 338),Pi-Pi stacked (Tyr341, Trp286), Pi-Akyl(Tyr341, Trp286),Pi-Pi T shaped (Trp86), and Alkyl(Val294) residue. According to the Ghose criteria for drug-likeness, molecular weight ranges from 160 to 480, computed log P ranges from −0.4 to 5.6.26, total number of atoms should range from 20-70, and molar refractivity ranges from 40 to 130(Ghose et al., 1999). Muegge criteria for drug likeliness include Hydrogen bond acceptor ≤ 10, Hydrogen bond donor ≤ 5, molecular weight between 200-600 Da, heteroatoms > 1,carbon atoms > 4, TPSA ≤ 150, XLOGP range -2 to 5, and the number of rings ≤ 7(Muegge et al., 2001). As evident in Table 1 of 15 compounds tested for drug likeliness only 6 compounds fulfilled the criteria for Muegge rule, while 8,9,10 and 10 compound fulfilled the criteria for Ghose, Lipinski, Egan, Veber respectively. In case of pharmacokinetic properties high GI Absorption was noted in 9 compounds, high BBB was noted in 6 compounds, 6 compounds have P-GP substrate, 2 compounds showed CYP1A2 and CYP2C9 inhibition, 3 showed CYP2C19 inhibition, 4 showed CYP3A4 Inhibiton and 5 compound showed CYP2D6 inhibiton(Table 3).

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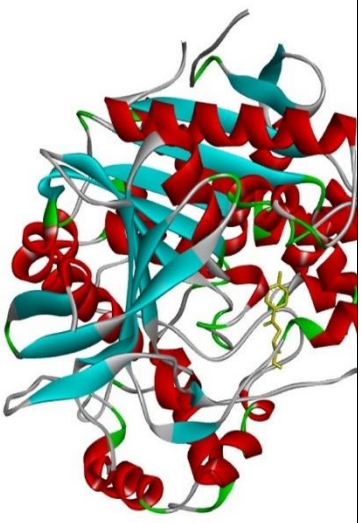
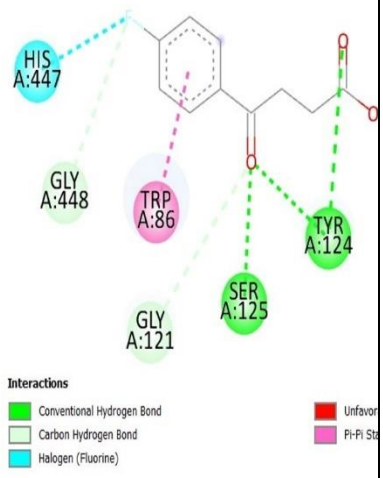
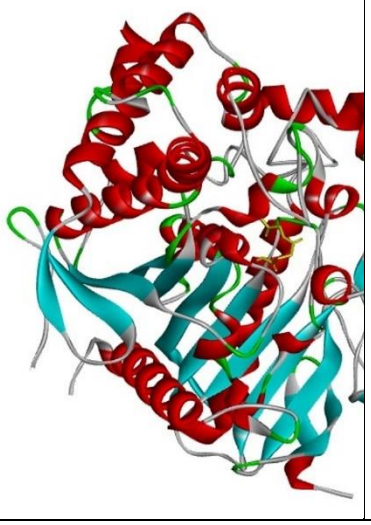
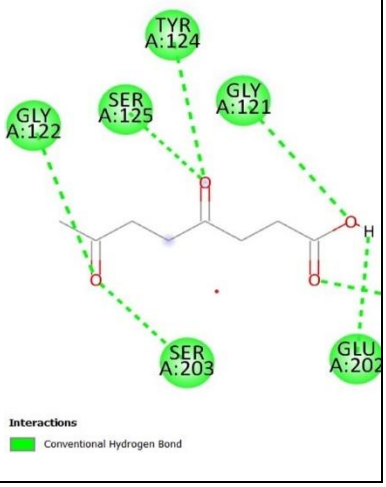
Table 1: Molecular Docking interaction of all phytochemicals with human acetylcholinesterase enzyme

S. No.	Compounds	PubChem ID	Binding energy (kcal mol−1)	Docking interactions	
				3D	2D
1.	3-(p-Fluorobenzoyl)-propionic acid	101359	-7.538		
2.	4,7-Dioxooctanoic acid	244084	-5.924		

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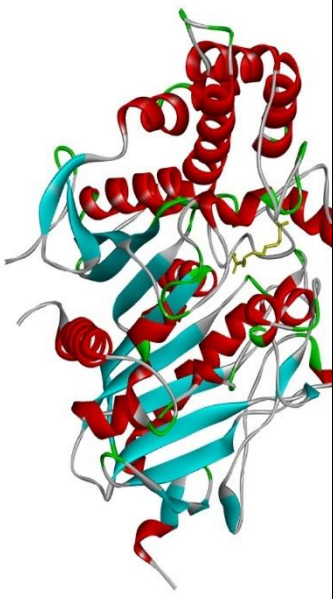
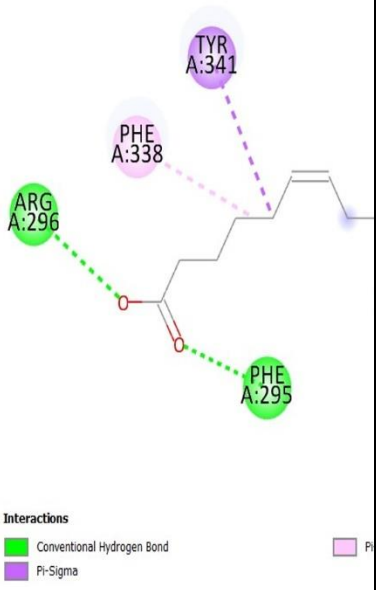
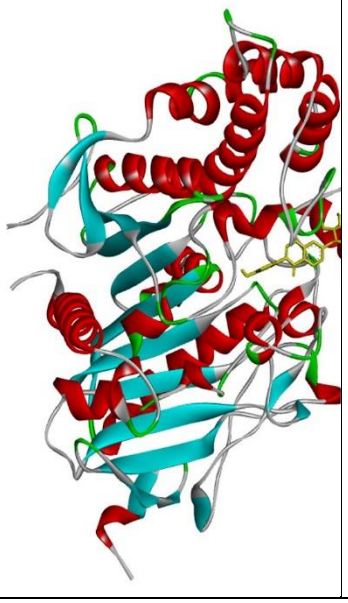
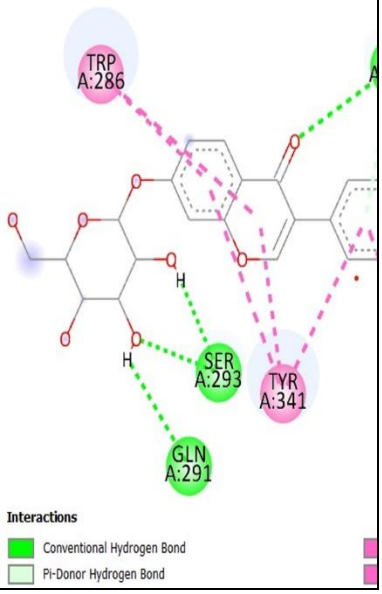
S. No.	Compounds	PubChem ID	Binding energy (kcal mol−1)	Docking interactions	
				3D	2D
3.	cis-4-Decenedioic acid	9543671	-6.8		
4.	Daidzin	107971	-11.306		

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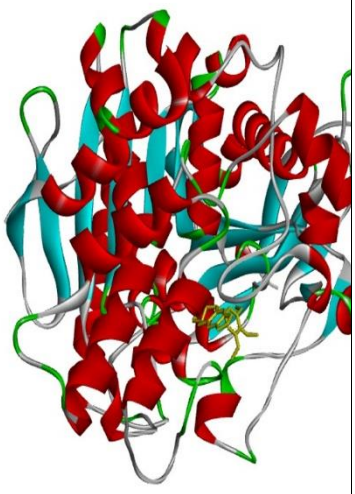
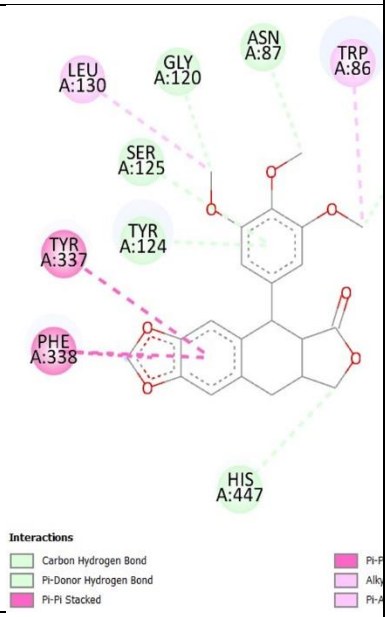
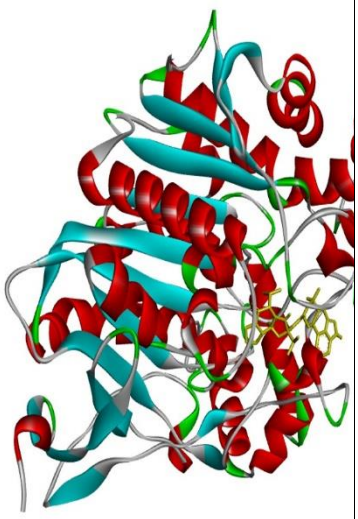
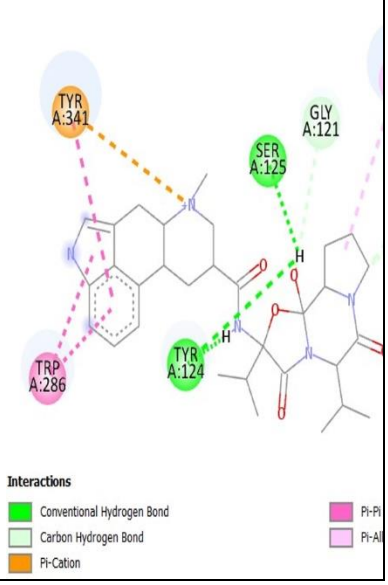
S. No.	Compounds	PubChem ID	Binding energy (kcal mol−1)	Docking interactions	
				3D	2D
5.	Deoxypodophyllotoxin	345501	-9.772		
6.	Dihydroergocornine	168871	-12.863		

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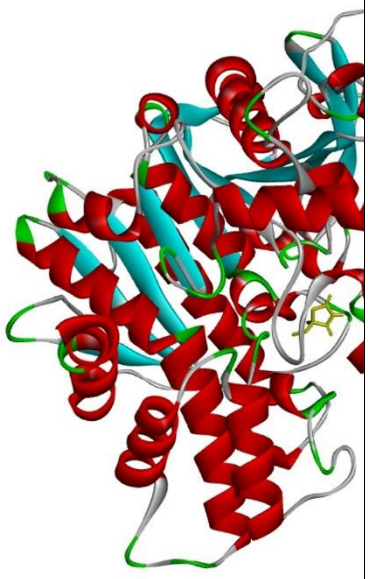
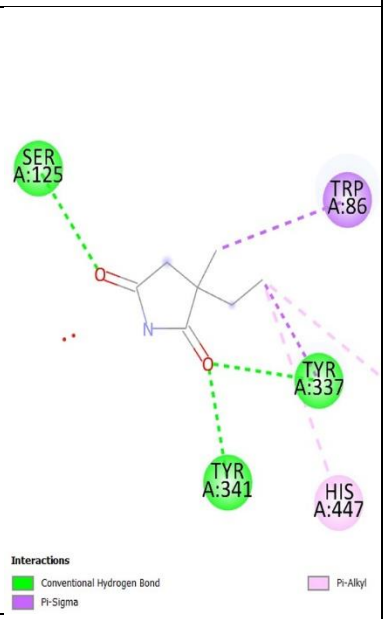
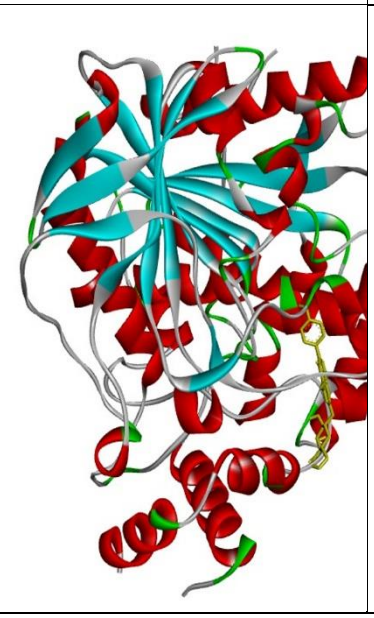
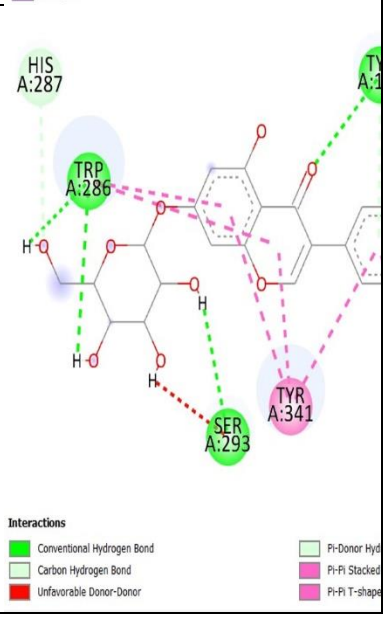
S. No.	Compounds	PubChem ID	Binding energy (kcal mol−1)	Docking interactions	
				3D	2D
7.	Ethosuximide	3291	-6.19		
8.	Genistin	5283177	-11.215		

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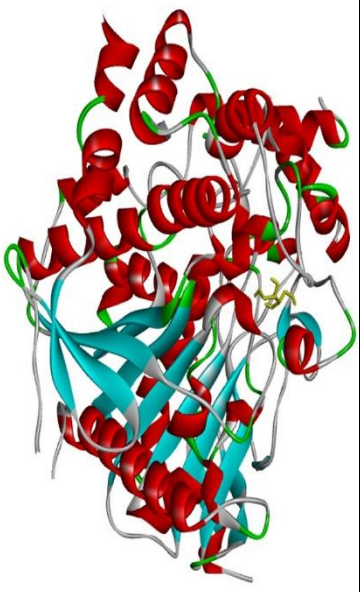
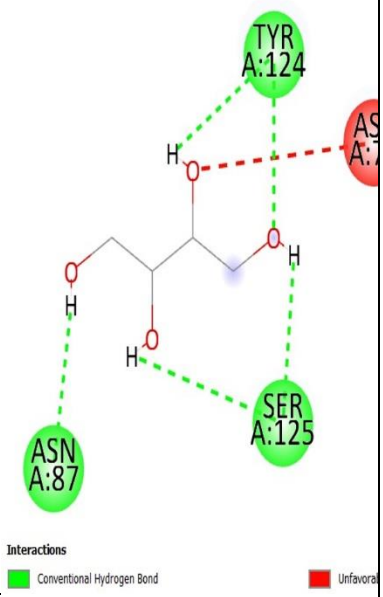
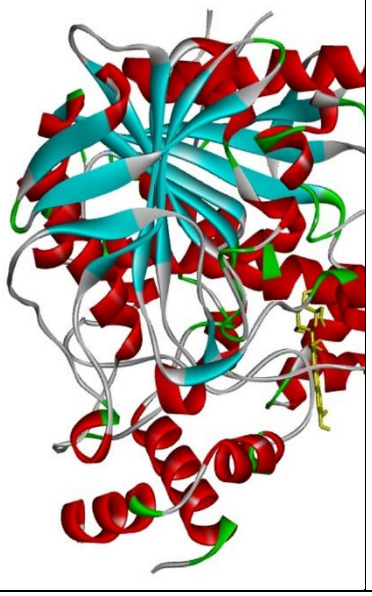
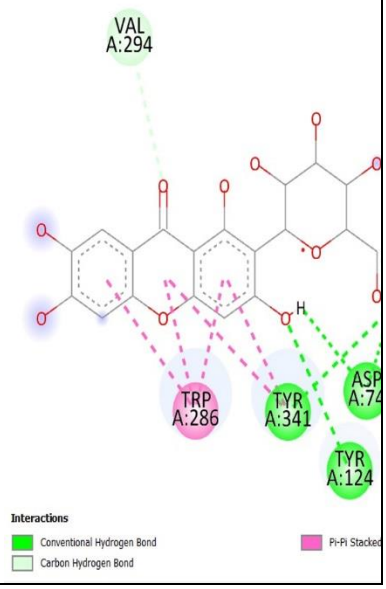
S. No.	Compounds	PubChem ID	Binding energy (kcal mol−1)	Docking interactions	
				3D	2D
9.	l-Threitol	445969	-4.64		
10.	Mangiferin	5281647	-9.318		

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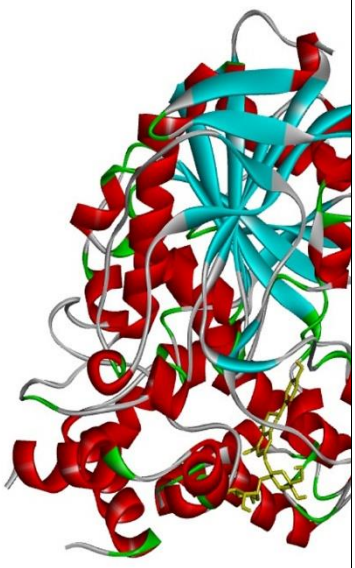
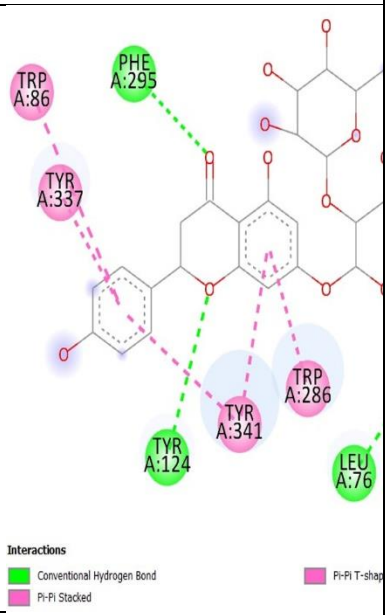
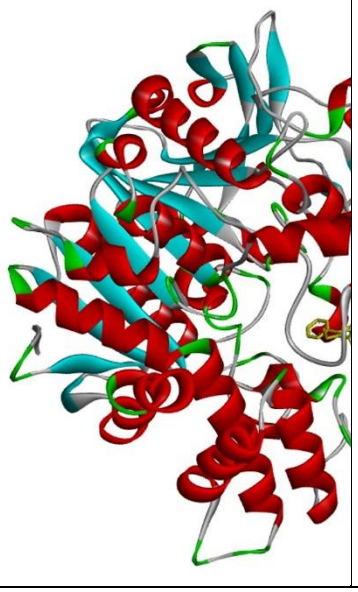
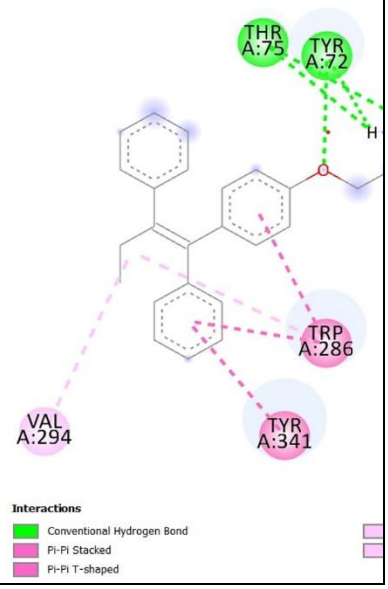
S. No.	Compounds	PubChem ID	Binding energy (kcal mol−1)	Docking interactions	
				3D	2D
11.	Naringin	442428	-11.538		
12.	N-Desmethyltamoxifen	6378383	-8.333		

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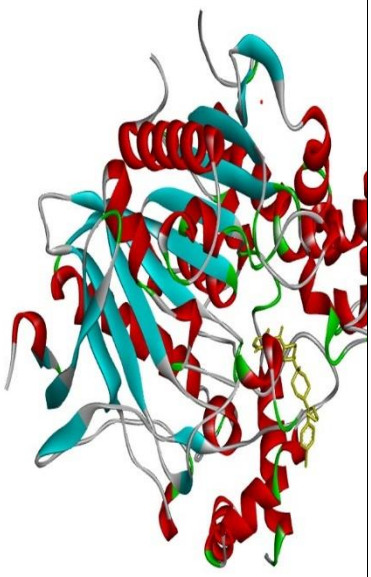
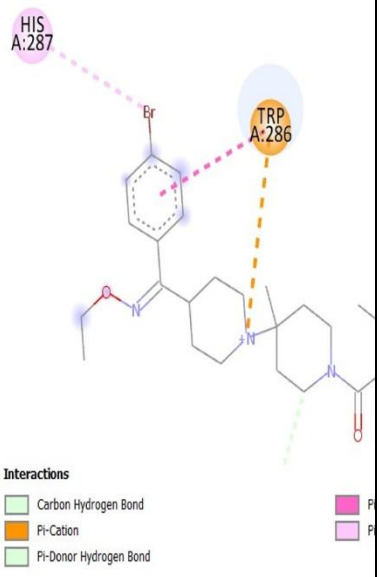
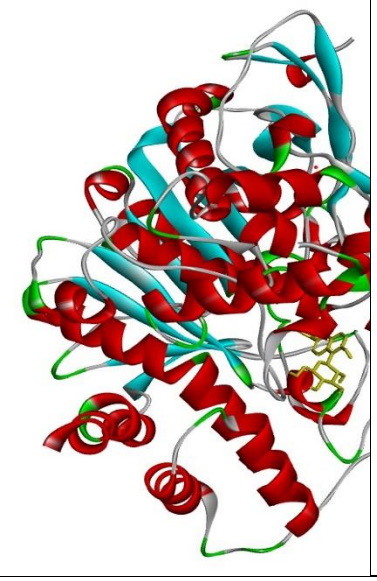
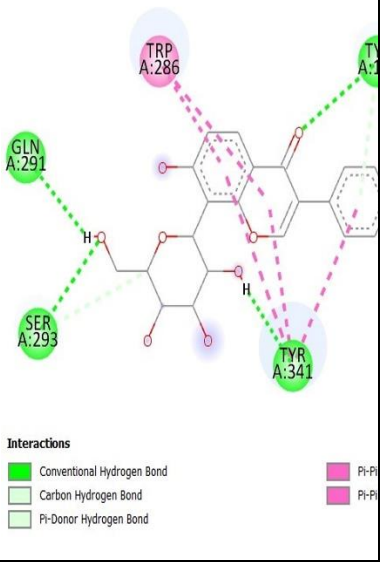
				3D	2D
13.	O-ethyloxime	9574343	-9.675		
14.	Puerarin	5281807	-10.159		

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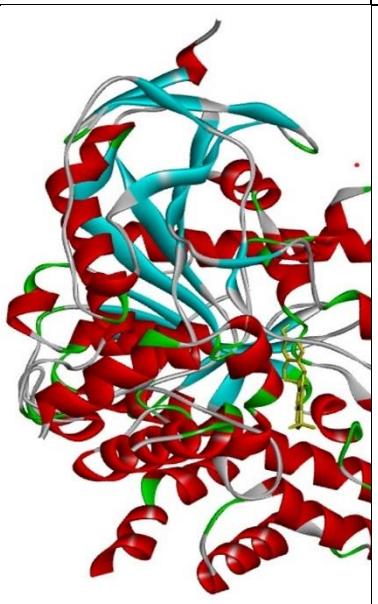
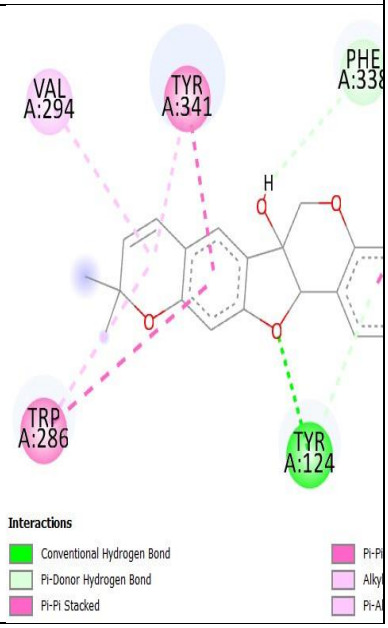
				3D	2D
15.	Tuberosin	14630495	-9.733		

Table 2: List of the results of the phytochemical molecules drug likeness properties.

S. No.	Compounds	Molecular weight (g/mol)	No. of H-bond acceptors	No. of H-bond donors	Molar Refractivity	Lipinski' RO5	Veber	Ghose	Egan	Muegge	Bioavailability score	TPSA (Å2)	No. of rotatable bonds	Solubility (mol/l)
1.	3-(p-Fluorobenzoyl)-propionic acid	196.18	4	1	47.98	Yes	Yes	Yes	Yes	No	0.85	54.37	4	8.70e-03
2.	4,7-Dioxooctanoic acid	172.18	4	1	42.74	Yes	Yes	Yes	Yes	No	0.85	71.44	6	1.14e+00
3.	cis-4-Decenedioic acid	200.23	4	2	53.25	Yes	Yes	Yes	Yes	Yes	0.85	74.60	8	3.95e-02
4.	Daidzin	416.38	9	5	104.09	Yes	No	Yes	No	Yes	0.55	149.82	4	1.06e-03
5.	Deoxypodophyllotoxin	398.41	7	0	102.68	Yes	Yes	Yes	Yes	Yes	0.55	72.45	4	4.81e-05
6.	Dihydroergocornine	563.69	6	3	164.77	No	Yes	No	Yes	Yes	0.55	118.21	5	3.04e-06
7.	Ethosuximide	141.17	2	1	40.51	Yes	Yes	No	Yes	No	0.55	46.17	1	1.29e-01
8.	Genistin	432.4	10	6	106.11	No	No	Yes	No	No	0.55	170.05	4	6.60e-04
9.	l-Threitol	122.12	4	4	25.99	Yes	Yes	No	Yes	No	0.55	80.92	3	1.11e+01
10.	Mangiferin	422.34	11	8	100.70	No	No	No	No	No	0.17	201.28	2	3.64e-03

11.	Naringin	580	14	8	134.91	No	No	No	No	No	0.17	225.06	6	1.04e-03
12	N-Desmethyltamoxifen	357.49	2	1	114.82	Yes	Yes	No	Yes	No	0.55	21.26	8	5.97e-07
13	O-ethyloxime	557.52	5	0	156.61	No	Yes	No	yes	yes	0.55	70.60	7	1.53e-06
14	Puerarin	416.38	9	6	104.59	No	No	Yes	No	No	0.55	160.82	3	2.37e-03
15	Tuberosin	338.35	5	2	91.84	Yes	Yes	Yes	Yes	Yes	0.55	68.15	0	3.89e-05

Table 3: The pharmacokinetic parameters of the identified compounds.

S. No.	Compounds	GI Absorption	BBB permeant	P-GP substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 Inhibitor	Log Kp (cm/s)
1.	3-(p-Fluorobenzoyl)-propionic acid	High	Yes	No	No	No	No	No	No	-6.42
2.	4,7-Dioxooctanoic acid	High	No	No	No	No	No	No	No	-7.99
3.	Cis-4-Decenedioic acid	High	Yes	No	No	No	No	No	No	-6.56
4.	Daidzin	Low	No	No	No	No	No	No	No	-8.36
5.	Deoxypodophyllotoxin	High	Yes	No	No	Yes	Yes	Yes	Yes	-6.52
6.	Dihydroergocornine	High	No	Yes	No	No	No	Yes	No	-7.09
7.	Ethosuximide	High	No	No	No	No	No	No	No	-6.89
8.	Genistin	Low	No	Yes	No	No	No	No	No	-8.33
9.	l-Threitol	Low	No	No	No	No	No	No	No	-8.67
10.	Mangiferin	Low	No	No	No	No	No	No	No	-9.14
11.	Naringin	Low	No	Yes	No	No	No	No	No	-10.15
12.	N-Desmethyltamoxifen	High	Yes	Yes	Yes	Yes	No	Yes	Yes	-3.74
13.	O-ethyloxime	High	Yes	Yes	No	No	No	Yes	Yes	-6.62
14.	Puerarin	Low	No	No	No	No	No	No	No	-8.83
15.	Tuberosin	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-5.98

Conclusion

All compounds isolated from tubers of *Pueraria tuberosa* showed significant binding energy with human acetylcholinesterase enzyme in molecular docking studies. Three compounds i.e, Deoxypodophyllotoxin, Tuberosin and cis-4-Decenedioic acid with a binding energy of -9.745,-9.733, and -6.8 kcal mol-1 are best suited candidate for devolvement as an Acetylcholinesterase inhibitor due to their high binding energies , Drug likeliness and favorable pharmacokinetics properties.

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Conflict of Interest:

The authors have no conflict of interest to declare.

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