Research Article

Molecular Docking Studies on Compounds Extracted from the Herbal Plant, *Evolvulus alsinoides* against Proteins involved in Alzheimer's Disease

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ABSTRACT

Alzheimer's disease is a form of dementia, in which nerve cells in memory areas of brain and eventually other areas begin to die at accelerated rate resulting in serious deterioration in several mental functions, such as loss in memory, language, orientation and judgment. These dementias could also have a devastating impact on developing countries, whose populations are aging most rapidly. By the year 2020, approximately 70% of the world's population aged 60 will be located in developing countries and it noted that 37 lakhs of Indians were already affected by Alzheimer's disease between 2002 to 2011. Further, it is predicted that AD will affect 1 in 85 people globally by 2050.According to Cholinergic hypothesis, AD is caused by reduced synthesis of the neurotransmitter ACh, wherein the AChE levels were increased which causes damage to the cholinergic neurons finally leading to cognitive impairments. Apart from this, a number of other changes such as extracellular deposits of β-amyloid senile plaques, intracellular formation of neurofibrillary tangles and the loss of neuronal synapses and pyramidal neurons occur culminating in further deterioration of neuronal functions. Even though a number of drugs are available as of today to treat AD, majority of them have exerted alarming side effects in patients. At this juncture, synthetic drugs have attracted the research community to design safe and effective drugs for AD. In the present study, an attempt to identify such natural neuroprotective compound was made on a set of 10 drug molecules from the plant, *Evolvulus alsinoides*, collected from PubChem Database. These compounds were docked against human AChE (P37136), Tau protein (4F2L), and APP proteins (3SUZ) retrieved from Protein Data Bank by Pyrex Virtual Screening tool (Auto Dock Vina). After performing docking, among these 10 compounds, the drug molecule kaempferol was found to have the highest binding energy i.e. -9.3, -6.1& -6.4 with all the target proteins viz. AChE (P37136), Tau protein (4F2L) and APP (3SUZ) respectively. Besides, it also contained the best binding affinity and interaction of amino acids on the active site of protein pocket binding region. Hence, kaempferol was predicted as the best drug molecule for prevention of AD.

Keywords: Alzheimers Disease, AChE, Tau protein and APP protein, Auto Dock Vina.

INTRODUCTION

Alzheimer's disease (AD), a common type of dementia affects a large portion of the elderly population over 65 years. Post-mortem of AD brain revealed three abnormal structures viz. Amyloid plaques clumps formed by the β-amyloid protein that accumulate outside the neurons and the Neurofibrillary tangled clumps of altered tau proteins inside the neurons. According to Cholinergic hypothesis, AD is caused by reduced synthesis of the neurotransmitter ACh, wherein the AChE levels were increased which causes damage to the cholinergic neurons finally leading to cognitive impairments. In AD patients, nerve cell damage typically begins with cells involved in learning and memory, and gradually spreads to cells that control every aspect of thinking, judgment and behaviour.

The current available drugs for treatment of Alzheimer's disease are not only symptomatic but do not alter the course or progression of the underlying disease and on the other hand produce adverse reactions in patients thereby limiting the scope for treatment of Alzheimer patients. Thus, there is need to develop targeted effective therapeutics for the treatment of Alzheimer's disease which may alter the course or progression of the underlying disease by preventing the formation or clearing of plaques (Beta-amyloid fragments). In recent years, many natural herbal treatments have been explored and the benefits derived from using herbal treatments for Alzheimer's and dementia have been proved very promising.

Evolvulus alsinoides, commonly known as shankhpushpi belongs to family Convolvulaceae and is considered as Medhya Rasayana in Ayurvedic texts for its boosting memory and improving intellect activities, and also in treatment of neurodegenerative diseases such as dementia, amnesia and also asthma[1]. Evolvine is an alkaloid, and the phytochemical kaempferol, isolated from Shankhpuspi is a potent radical scavenger and efficient antioxidant[2]. Besides, it also contains many alkaloids viz., betaine, sankhapushpine and evolvine, scopoletin, scopolin, umbelliferone, 6-methory-7-0-ß-glucophyranoside coumarinqueretine-3-o-ß-glucophyrenoside[3].

The compounds isolated E. alsinoides aerial parts have been elucidated for the first time on the basis of spectral data analysis and chemical reactions. However, molecular Docking Studies on Compounds from *Evolvulus alsinoides* against proteins involved in Alzheimer's disease have not been made so far. The present work is one such an attempt to design drug compound against Alzheimer's

disease from the lead molecules of *Evolvulus alsinoides* plant since this plant extract also exhibited antioxidant and immunomodulatory properties[4] and also key role in progression of AD.

MATERIALS AND METHODS

Present work was mainly focused on three proteins viz. AChE Proteins associated with Alzheimer's disease. Tau protein and Amyloid Precursor Protein (APP) sequences were retrieved from NCBIby using URL www.ncbi.nlm.nih.gov and the 3D Structures for these proteins downloaded from PDB by using URL http://www.rcsb.org/PDB. Sequence of rat Acetylcholinesterase (AChE) enzyme was retrieved from UniPort and submitted to Modeller 9V10 for 3D Structure Modelling. Ligands against these proteins were selected from the plant, Eolvulus alsinoides[3] and they were retrieved from PubChem database. Argus Lab, Cast-P server, Auto Dock vina (Pyrx), Pymol, Osiris, Pass Prediction are the software's and Bioinformatics tools employed in the present work.The docking studies are helpful for understanding the binding mode and interaction of three proteins with ten ligands. The three proteins Tau protein, APP protein and AChE were docked with the ligands from the plant *Evolvulus alsinoides*. For the docking of ligands into protein active site and to estimate the binding affinities of docked compounds, an advanced molecular docking program AutoDock Vina (1.1.2) was used in this study. The rotational bonds of the ligands were treated as flexible while those of the protein were kept rigid. Grid boxes were fixed around an active site of the protein.

Preparation of ligands:

The 10 drug molecules from the plant, *Evolvulus alsinoides* were collected in 3D SDF format from PubChem database http://puBChEm.ncbi.nlm.nih.goc/. The compounds were added hydrogen's and energy minimized with UFF force field using conjugate- gradient algorithm by Auto Dock and Pyrex 20. Later, all lead molecules were converted in to Auto Dock Pdbqt format.

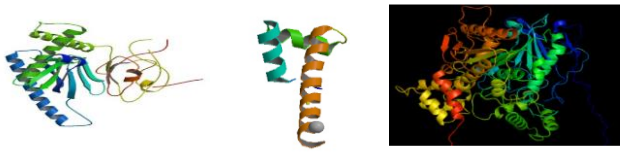
Preparation of Proteins for docking studies:

The Tau protein (4F2L) & APP (3SUZ) proteins, retrieved from Protein Data Bank were opened with word document and removed hetero atoms then energy minimization was performed by using Argus lab.

RESULTS

PDB Structures of selected Proteins

3SUZ **4F2L** **P37136**



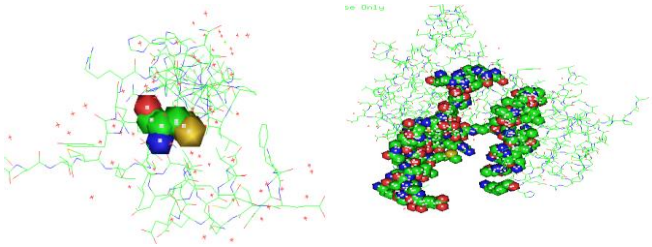
Ligands selected for docking studies from *Evolvulus alsinoides*

2-cmethyl erythritol	Sitosterol	Scopolin	Caffeic acid	Scopoletin
Kaempferol	Umbelliferone	Octyl octadeceonate	Tropane	B Stigmasta

Identification of Active site for selected proteins

The Pymol interaction of selected drug molecules on the binding pocket of three proteins active sites were predicted by using (CASTp) program (http://cast.engr.uic.edu)[5].

4F2L through CAST-P **3SUZ through CAST-P**



From the CAST-p, we predicted that the following amino acid residues were present at the proteins pocket binding site.

TYR 378, LEU 379, GLY 380, SER 381, TYR 382, GLN 383, ARG 405, VAL 406, MET 409, GLN 410, ALA 412, ALA 413, LYS 416, LYS 417, ALA 419, ASN 420, SER 421, GLU 422, GLY 423, ASP 424, ALA425, GLN 42, TYR 427, LEU 428, TUR 429, ARG 439, ASN 444, GLU 449, TYR 450, MET451,MET 452, ASP 453, HIS 454, ALA 455, ARG 457, TYR 458, ALA 473, ARG 474, ARG 475, ARG 476, MET 477, LYS 490, ILE 492, HIS 494, GLU 553, ILE 560, LYS 577, ILE 623, ASN 624, GLN 625, TYR 626, SER 627, LEU 628, VAL 629, GLY 630, LEU 631, ALA 634, TYR 635, GLY 630, ILE 639, GLY 642, LEU 643, ASN 645, GLN 646, GLN 648, LYS 650.

Prediction of drug likeness properties by using OSIRIS

The OSIRIS Property Explorer is to draw chemical structures and calculate on-the-fly various drug-relevant properties whenever a structure is valid. Prediction results are valued and colour coded. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red, whereas green colour indicates drug-conform behavior.

Out of ten lead molecules, the best 5 lead molecules were selected based on binding affinity values for Osiris. Among these five molecules, it was evident that Kaempferol showed the best drug-related properties such as Solubility, Drug Likeness, and Drug Score etc.

Table 1: Protein and ligand docking Visualization by Pymol:

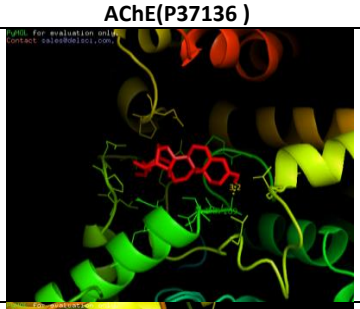
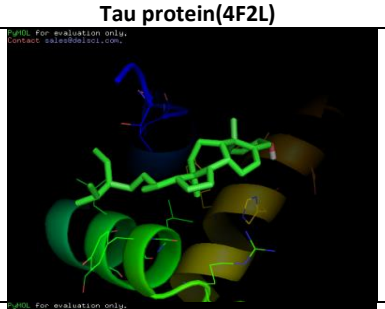
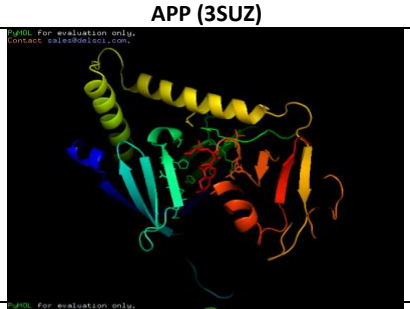
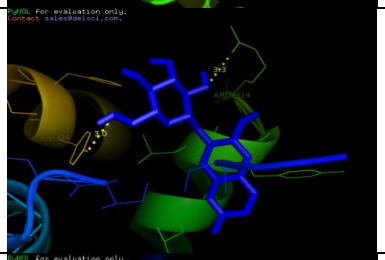
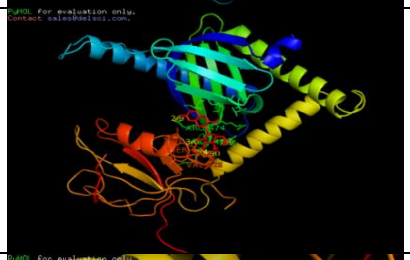
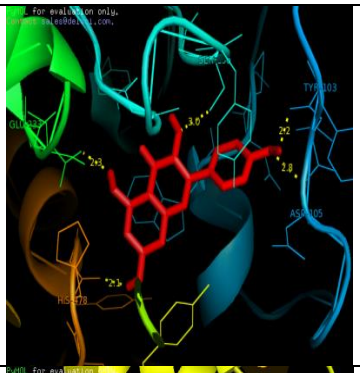
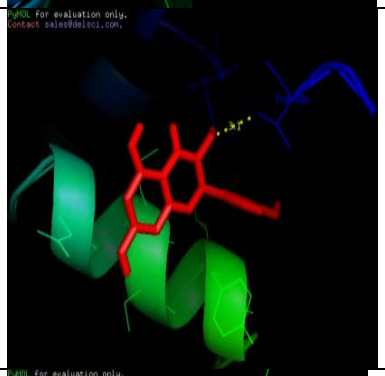
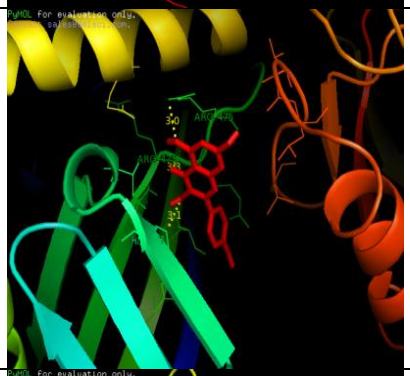
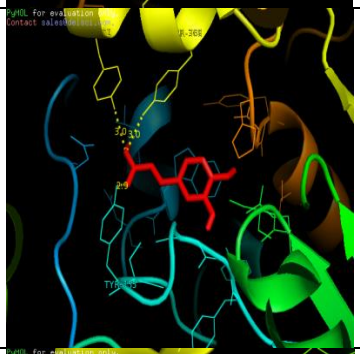
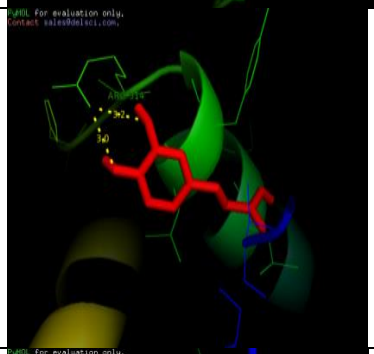
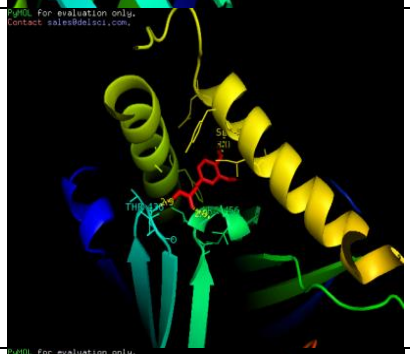
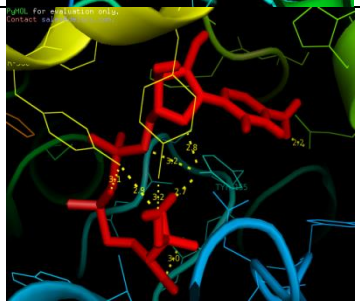
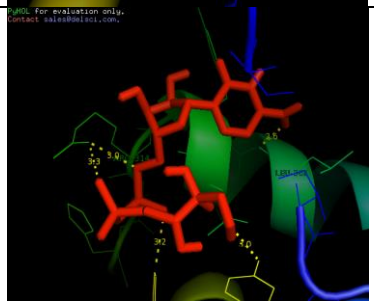
Ligands	AChE(P37136)	Tau protein(4F2L)	APP (3SUZ)
B-Sitosterol			
Scopolin			
Kaempferol			
Caffeol quinic acid			
2-c Methyl Erythritol			

Table 2: Showing Interaction Studies between the Protein AChE (P37136)-Ligand through Pymol

Ligand	Hydrogen bonding			Distance	Binding affinity
	Residue	Atom	Ligand		
B-Sitosterol	THR 269	O	H	3.2	-7.7
Scopolin	TYR 269	O	O	2.8	-8.4
	SER 156	O	O	2.8	
	SER 156	O	O	3.3	
	GLY 151	O	H	2.4	
	GLY 151	N	O	3.2	
	TYR 164	O	O	3.3	
	TYR 164	O	H	2.8	
Kaempferol	TYR 103	O	H	2.2	-9.3
	ASP 105	N	H	2.8	
	SER 156	O	O	3.0	
	GLU 233	O	H	2.3	
	HIS 478	O	H	2.1	
Caffeolquinic acid	TYR 368	O	O	3.0	-7.4
	TYR 155	O	O	2.9	
	TYR 372	O	O	3.0	
2-C Methyl Erythritol	GLU 316	O	H	2.2	-8.6
	TYR 155	O	O	2.8	
	TYR 155	O	O	3.2	
	TYR 155	O	O	2.7	
	TYR 372	O	O	3.2	
	TYR 368	O	O	2.9	
	TYR 368	O	O	3.1	

Table 3: Showing Interaction Studies between the TauProtein (4F2L) -Ligand through Pymol:

LIGAND	Hydrogen bonding			Distance	Binding affinity
	Residue	Atom	Ligand		
β-Sitosterol	-	-	-	-	-6.5
Scopolin	ARG 314	N	O	3.3	-5.5
	TYR 324	O	O	3.0	
Kaempferol	THR 286	O	O	3.1	**-6.1**
Caffeol quinic acid	ARG 314	N	O	3.3	-5.5
	ARG 314	N	O	3.0	
2-c Methyl Erythritol	ARG 314	N	O	3.0	-5.4
	ARG 314	N	O	3.3	
	HIS 325	N	O	3.2	
	TYR 324	O	H	3.0	
	LEU 308	O	H	2.6	

Table 4: Showing Interaction Studies between the APP Protein (3SUZ) -Ligand through Pymol

LIGAND	Hydrogen bonding			Distance	Binding affinity
	Residue	Atom	Ligand		
β-Sitosterol	-	-	-	-	-7.4
Scopolin	ARG 475	N	O	2.9	-7.1
	VAL 628	O	H	2.0	
	VAL 628	O	H	2.3	
	SER 626	O	O	3.4	
	SER 626	N	O	3.0	
	ARG 474	N	O	2.9	
Kaempferol	PHE 521	O	H	2.2	-6.4
	HIS 453	N	O	3.1	
	ARG 473	O	O	3.3	
	ARG 475	N	O	3.0	
Caffeic acid	THR 436	O	H	2.9	-6.5
	ARG 456	N	O	2.9	
	SER 545	O	O	3.0	

2-c Methyl Erythritol	ASP 452	N	O	2.8	-6.7
	ASP 452	O	H	2.6	
	HIS 453	N	O	3.0	
	ARG 475	N	O	3.4	
	ARG 475	N	O	2.9	
	ARG 475	N	O	2.9	
	ARG 474	N	O	3.3	
	SER 626	N	O	2.9	
	SER 626	O	O	3.2	
	VAL 628	O	H	2.0	

Table 5: showing Data generated for selected Ligands by Osiris

Name	Compound id	C log p	Solubility	Mol. wt	DLN	Drug score
Kaempferol	5280863	2.1	-2.79	286.0	0.9	0.22
b-Sitosterol	222284	8.24	-6.84	426.0	-4.3	0.1
Caffeoylquinic acid	1794427	-0.44	-1.5	354.0	0.17	0.7
Scopolin	439514	-0.56	-1.98	354.0	-5.11	0.45
2-C methyl erythritol	24970669	-5.18	-1.34	462	-29.88	0.24

PASS Prediction

PASS (Prediction of Activity Spectra for Substances), a tool for evaluating the general biological potential of an organic drug-like molecule provides simultaneous predictions of many types of biological activity based on the structure of organic compounds. Thus, PASS can be used to estimate the biological activity profiles for virtual molecules, prior to their chemical synthesis and biological testing.

DISCUSSION

In the present study, the results generated by AutoDock Vina revealed that Kaempferol showed the highest binding affinity values such as -9.3,-6.1&-6.4 with AChE, Tau (4F2L) and APP protein (3SUZ) in that order (Table.2,3&4). Protein-ligand Interaction and Visualization by Pymol showed that the selected lead molecules exhibited the best interaction with the following amino acids viz. TYR 103, ASP 105, SER 156, GLU 233, HIS 478 in the binding pocket of AChE, THR 286 in the binding pocket of 4F2L and ARG 475, VAL 628, VAL 628, SER 626, SER 626, ARG 474 in the binding pocket of 3SUZ. However, β-Sitosterol and Stigmasta-5 showed the highest binding affinity value (-6.5) with 4F2L but they didnot show interactions with the protein. Similarly, Stigmasta-5 also showed highest binding affinity (-7.5) with the 3SUZ but it doesn't show any interactions as seen from PyMol visualization (Table.1).

As a corollary to the above, results obtained from the Osiris output, revealed that Kaempferol showed the best drug related properties such as Solubility, Drug Likeness, Drug Score etc. Kaempferol, the C log P value is 2.1, Solubility is -2.79, Mol.wt is 286 Dal, DLN is 0.9, Drug score is 0.22 (Table.5). As such, it is obeying all the rules of Osiris. Further, we found that Kaempferol did not show any mutagenic properties when compared to other lead molecules.

By and large, Kaempferol has many important biological properties but only very important points such as Beta amyloid protein antagonist, Kinase inhibitor, Antioxidant, Neurotrophic factor enhancer, Huntington's disease treatment, Antitoxic, Amyloid beta aggregation inhibitor, Nootropic, Cholesterol absorption inhibitor, Apoptosis antagonist, Vascular dementia treatment etc were performed by using PASS prediction tool (Table.6).

Kaempferol, by virtue of being a flavonoid phenolic compound exist widely in fruits, vegetables, and plants including some herbal medicines. Flavonoids have a wide variety of biological activities and therapeutic potential6. Several flavonoids, including apigenin, chrysin, wogonin, baicalein, and baicalin, have been shown to have positive behavioural effects on rodent EPM and VCT with an anxiolytic potency comparable to that of typical BDZ agents7. Pre-treatment with specific BDZ receptor antagonists largely abolished the anxiolytic effects of flavonoids. Several flavonoids were capable of binding to GABA-A receptors with significant affinity8. Therefore, flavonoids seem to exert their behavioural effects through modulation of the GABA-A receptor complex. Besides, Flavonoids also possess potent neuroprotective activities 9, 10.

Nevertheless, the search for potent and long-acting Acetylcholinesterase (AChE) inhibitors that exert minimal side effects in AD patients is still ongoing. As on today, AChE inhibitors are the only approved therapy for the treatment of Alzheimer's disease.

CONCLUSION

Based on the results obtained on the docking studies in the present study and from the biochemical estimations already carried out by the present researchers, *Evolvulus alsinoides* may be suggested as a potential therapeutic drug for treatment of AD. However, there is a need to carry further investigations with the help of the insilico

approach to generate more effective and potential drug through ligand-based drug designing approaches.

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