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Neuroprotective Effects of Secondary metabolites as a inhibitor of Acetylcholinestrase (Ache) for treatment of Alzheimer's Disease : An *in silico* Approach

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KEYWORDS
Ache – Acetylcholinesterase,
AD- Alzheimer's Disease,
NDD- Neurodegenrative disease,
ADME – Absorption, Distribution, Metabolism, Excretion.

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ABSTRACT

Neurodegenerative disorders (NDD) constitute a series of pathological conditions which originating from slow progressive and irreversible dysfunction and can lead to loss of neurons and synapses in selected areas of the brain which determine clinical presentation and course. Common neurodegenerative disorders are Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, Parkinson's disease , frontotemporal dementia and the spinocerebellar ataxias. The study was designed to find the Effects of secondary metabolites as a potent inhibitor of Acetylcholinestrase (Ache) by in silico approach with help of Lipinski And ADME analysis for treatment of Alzheimer's Disease. Out of 100 compound , find 5 compound Alpha-lapachone, Harmaline ,Harmine, Daidzein, Isopimpinellin are actively inhibit the Activity of Ache out of five, "Daidzein" is most effective inhibitor of Ache according to Docked Score compared to Donepezil and Galantamine Which are already present for treatment of Alzheimer's Disease.

INTRODUCTION

Neurodegenerative disorders (NDD) constitute a series of pathological conditions which originating from slow progressive and irreversible dysfunction and can lead to loss of neurons and synapses in selected areas of the brain which determine clinical presentation and course. Themajor basic mechanisms which is leading to neurodegeneration (ND) are depend upon many factors caused by genetic, environmental and endogenous factors related to aging, but their pathogenic role and their basic molecular mechanisms are not fully understood (Jellinger KA2009)(Skovronsky DM, Lee VM-Y et al 2006). NDDs are classified according to their known genetical mechanisms and major compounds of their protein deposition. Based oncritical conformational changes of proteins, these disorders are denoted as 'protein misfolding' diseases or protein pathies (Golde TE, Miller VM 2009) (Uversky VN 2009).

Neurodegenerative disorders are major threat to human mental health. Neurodegenerative disorders are age dependent and are becoming increasingly prevalent, in part because the elderly population has increased in recent years (Heemels, 2016. Common neurodegenerative disorders are Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, Parkinson's disease , frontotemporal dementia and the spinocerebellar ataxias. These disorders have different mechanism in their pathophysiology – with some causing memory and cognitive impairments and others affecting a person's ability to move, speak and breathe (Abeliovich and Gitler, 2016; Canter et al., 2016; Taylor et al., 2016; Wyss-Coray, 2016)

Alzheimer's Disease:

Alzheimer disease (AD) is the most common neurological disorder in which neurodegenerative takes place. It is a menacing, progressive and degenerative disease.AD is the very common cause of the dementia. Dementia can leads to the loss of cognitive functioning—like thinking, remembering, reasoning— behavioral abilities of brain and it also can cause death. In studies it is estimated that till 2050 around 11 million people will be influenced by the Alzheimer's disease (Alzheimer's, 2014, 2015). AD is named after Dr. Alois Alzheimer. In 1906, Dr. Alzheimer saw some changes in the neuronal tissue of a woman who had died of an unknown and unusual mental illness. She have some symptoms like loss of memory, problem in communicating , and unpredictable behavior. After her death, Dr. Alzheimer examined her brain and found many abnormal aggregated clumps and tangled fibers. These abnormal clumbs which are now known as plaques and tangles in the brain tissues are considered some of the main features of Alzheimer's disease. Another feature of AD is the the loss of connections between neuronal cells in the brain. exact reason and pathophysiology of AD is still unclear and there is a no known cure of this disease . (Mattson, 2004).

Acetylcholinesterase:

Acetylcholinesterase is an enzyme which is participate in cholinergic neuronal transmission. It break acetylcholine which terminates the neurotransmission mechanism (Ballard et al 2005).

AChE activity is repressed by many compounds. The number of known inhibitors have wide range. There are two main types of inhibitors can be illustrious from a practical point of view: toxins and drugs (Schwarz, M 1995).The inhibitors are compounds with different structural and functional motives as they can bind to the esteratic part of the active site by esterification of serine hydroxyl, or act together with the alpha anionic part of the active site, the aromatic gorge and the peripheral anionic site (Weiner, L et al 2009). Assay of AChE activity can serve for diagnosis after potential exposure to organophosphorus or carbamate pesticides and nerve agents(Lessenger, J.E 1999)(Bajgar, J 2004). It can also be used for verification of treatment efficacy, e.g., for Alzheimer's disease therapy (Sramek, J.2000). Novel drugs for Alzheimer's disease or antidotal therapy are tested by in vitro methods when AChE is mixed up in the treatment process (Guo, A.J et al 2010)(Hoskovcova et al 2009). Assay of nerve agents and selected pesticides by devices with AChE is another application of this enzyme(Pohanka, M et al2009). . Experimental methods for Acetylcholinesterase activity assay have been proposed.regrettably, the mechanism of AChE activity assay had boundaries that can prevent its use in some pharmacological or toxicological experiments. The most frequent assay is based on Ellman's way using an alternative substrate acetylthiocholine and 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB). The reaction lead to production of 5-thio-2-nitrobenzoate that has yellow color due to the shift of electrons to the sulfur atom. The method was developed by Ellman and coworkers in the early 1960s(Ellman, G.L et al 1961) and it is still used up to now, generally with significant changes (Pohanka, M. et al 2008). The Ellman's system is particularly restricted for testing antidots against organophosphorus AChE inhibitors or for measuring AChE activity insamples of such treated individuals. The antidots contain reactive oxime group splitting DTNB and provide false positive reaction in a process called oximolysis(Pohanka, M et al . 2008).

MATERIALS AND METHODS:

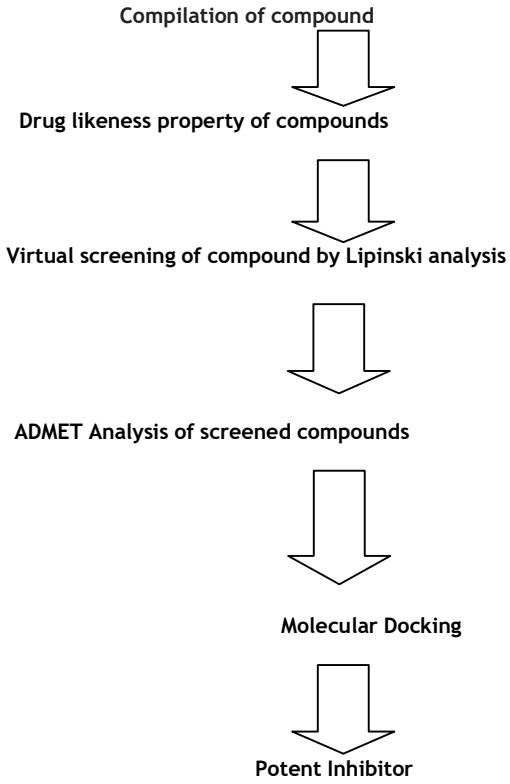
Online Tools-

1. Pubchem (https://pubchem.ncbi.nlm.nih.gov).
2. RCSB PDB (https://www.rcsb.org).
3. Molinspiration (https://molinspiration.com/cgi-bin/properties).
4. PreADMET Online Prediction Tool (https://preadmet.bmdrc.kr/adme/).

Offline Tools-

1. AutoDock 4.2.6.
2. Cygwin.
3. Discovery Studio 2020 Client.
4. Pymol.
5. Openbabel.

METHODS:-



Preparation of Compounds-

1.1.Target protein preparation-

The tertiary structure of the protein target is retrieved from the RCSB protein database. Accelery Discover Studio tool is used to prepare the targets such a way that it has no ambiguities i.e. water molecules and small molecules along with the ligand/substrate and any HETATM attached are removed from the primary downloaded complex.

Table No.1- Crystallographic Properties of the Target

RCSB ID	Classification	Organism	Expression System	Res.	Method	Mutation
4EY7	Hydrolase	Homosapiens	homosapiens	2.35 Å	X-ray diffraction	NO

Figure 1:4EY7

Ligand preparation-

From the studies we have taken the reported bioactive compounds for our *in-silico* study (Table 2). The three-dimensional structures of these compounds are retrieved from the PUBCHEM open chemistry database.

Table No.2- Compiled Compounds with their PubChem ID and their Chemicalformula.

S.No.	Compounds	PubChem Id
1.	Alpha-Lapachone	7273
2.	Daidzein	5281708
3.	Harmaline	3564
4.	Harmine	5280953
5.	Isopimpinelliin	68079

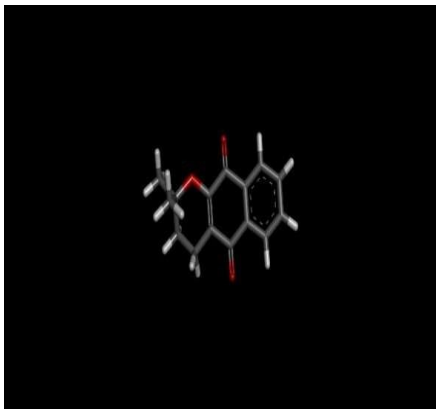


Figure 2: Alpha-lapachone

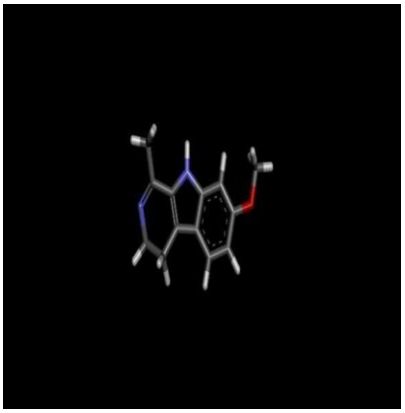


Figure 3: Harmaline

Figure 4: Daidzein

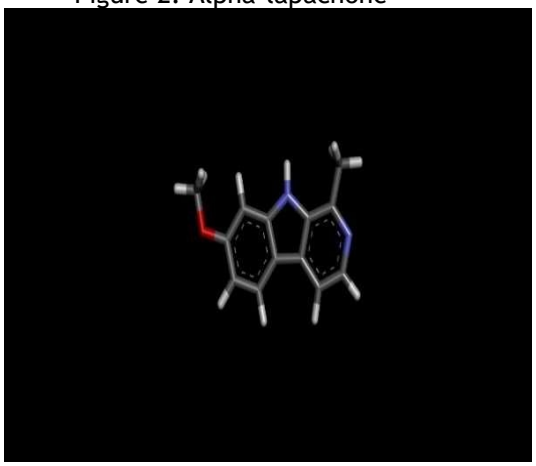


Figure5:Harmine

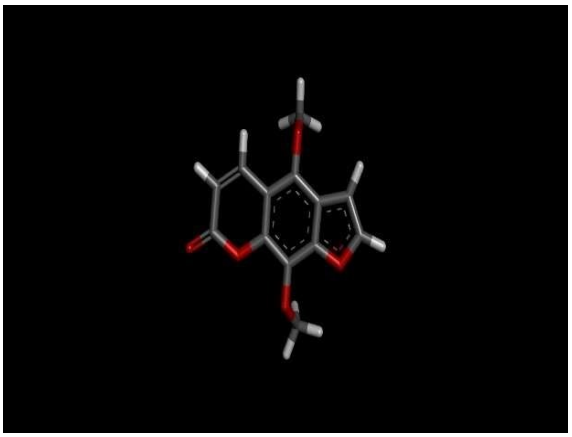


Figure 6:Isopimpinellin

Reference compounds preparation-

Alzheimer's drugs is a strategy to help the temporarily management of memory loss, thinking and reasoning problems, and day-to-day function. Regrettably, Alzheimer's drugs do not show their effectiveness on everyone, and they can't treat the disease or stop its continuous progression. Over time, their effects disappears
The Food and Drug Administration (FDA) has approved two types of drugs specifically to treat symptoms of Alzheimer's disease.

- Cholinesterase inhibitors
- Memantine

Two cholinesterase inhibitors are commonly prescribed:

- **Donepezil (Aricept)** is used for the treatment of all stages of the AD disease. It's takenonce a day in a tablet form.
- **Galantamine (Razadyne)** is approved for the treatment of mild to moderate AD . It'staken in a tablet form once a day or as an extended-release capsule twice a day.

Table No.3- Standard Compounds with their PubChem ID and Chemical formula.

S.No.	Standard	PubChem ID	Chemical Formula
1.	**Donepezil**	3115	C24H29NO3
2.	**Galantamine**	9651	C17H21NO3

Pharmacological activity study-

"Drug-Likeness" study-

This study helps to improve the prediction and estimated bioavailability of leading ligands and membrane penetrability 'the Lipinski lead of 5' is applied here. It helps to calculate the absorption of the compounds by relying on their molecular weight (MW), log P (segmentcoefficient), or the distribution of donor and acceptor hydrogen-bonds (Lipinski CA,2004).Drug-Likeness analysis was done using an online server tool called Molinspiration. (https://www.molinspiration.com/cgi-bin/properties).

Table 4:Drug-likeness property of compiled compounds

S. No.	Compounds Name	XLogP3	Mol Wt. (g/mol)	Hydrogen Bond Donor Count(nOH)	Hydrogen Bond Acceptor Count(nOHNH)	N violation
Reference/Standard Compounds						
1.	Donepezil	4.10	379.50	4	0	0
2.	Galantamine	1.54	287.36	4	1	0
COMPOUNDS						
1.	Agroclavine	3.00	238.33	2	1	0
2.	Ajmaline,	2.56	326.93	4	2	0
3.	Alpha pinene	3.54	136.24	0	0	0
4.	Alpha-lapachone	2.95	242.27	3	0	0
5.	ALPHA-	2.16	152.24	1	0	0

	thujone					
6.	Alstonine	3.54	348.40	5	0	0
7.	Anthraquinone	3.67	208.22	2	0	0
8.	Apigenin	2.46	270.24	5	3	0
9.	Aromoline	5.96	594.71	8	2	2
10.	Asarone	2.49	208.26	3	0	0
11.	Asiatic acid	-4.70	488.71	5	4	0
12.	Berberine	0.20	336.37	5	0	0
13.	Betaine	-5.41	117.15	3	0	0
14.	Betulinic acid	7.04	456.71	3	2	1
15.	Boscartin C	3.30	338.49	4	02	0
16.	Bufotenine	1.79	204.27	3	2	0
17.	Butanol	0.92	74.12	1	1	0
18.	Butyric acid	-1.00	88.11	2	1	0
19.	Calotoxin	0.45	548.63	10	4	1
20.	Cathine	0.33	151.21	2	3	0
21.	Centelloside	0.30	828.99	16	11	3
22.	Cimigenol	4.76	488.71	5	3	0
23.	Cineole	2.72	154.25	1	0	0
24.	Corynoxeine	2.60	382.46	6	1	0
25.	Coumarin	2.01	146.15	2	0	0
26.	Curcumin	2.30	368.38	6	2	0
27.	Dactylorhin B	-2.58	904.87	23	13	3
28.	Daidzein	-2.56	254.24	4	2	0
29.	Epidherine	1.24	165.24	2	2	0
30.	Erucic acid	8.96	338.58	2	1	1
31.	Eugenol	2.10	164.20	2	1	0
32.	Fisetin	1.97	286.24	6	4	0
33.	Galanthamine	1.54	287.36	4	1	0

34.	Gingerol	3.22	294.39	4	2	0
35.	Ginkgolide-B	-2.38	424.40	10	3	0
36.	Glycyrrhizin	1.97	822.94	16	8	3
37.	Gomisin D	4.11	530.57	10	2	1
38.	Harmaline	2.68	214.27	3	1	0
39.	Harmine	2.63	212.65	3	1	0
40.	Honokiol	5.00	266.34	2	2	1
41.	Huperzine A	2.65	242.32	3	3	0
42.	Huperzine b	2.97	256.35	3	2	0
43.	Hydroxybenzyl isothiocyanate	2.53	165.22	2	1	0
44.	Hyoscine	1.05	303.36	5	1	0
45.	Isopimpinellin	2.26	246.22	5	0	0
46.	Isovitexin	0.52	432.38	10	7	1
47.	Jujuboside A	-0.47	1207.36	26	14	3
48.	Kaempferol	2.17	286.24	6	4	0
49.	Lanosterol	8.48	426.73	1	1	1
50.	Limonene	3.62	136.24	0	0	0
51.	Limonin	-2.53	470.52	8	0	0
52.	Linalool	3.21	154.25	1	1	0
53.	Linamarin	-1.43	247.25	7	4	0
54.	Linoleic acid	6.86	280.45	2	1	1
55.	Lochnerine	3.31	324.42	4	2	0
56.	Lochnerine	3.31	324.42	4	2	0
57.	Lotaustralin	0.93	261.27	7	4	0
58.	Lupeol	8.29	426.73	1	1	1
59.	Lycorine	0.54	287.31	5	2	0
60.	Madecassoside	-0.55	975.13	20	13	3
61.	Mescaline	0.55	211.26	4	2	0

62.	Militarine	-0.27	726.73	17	9	3
63.	Mucilage	-6.38	1691.48	50	16	3
64.	Muscarine	-3.81	174.26	3	1	0
65.	Nicotine	1.09	162.24	2	0	0
66.	Nimbolide	1.94	466.53	7	0	0
67.	Nornicotine	0.85	148.21	2	1	0
68.	Palmatine	-.05	352.41	5	0	0
69.	P-cymene	3.90	134.22	0	0	0
70.	P-cymene	3.90	134.22	0	0	0
71.	Peonidin	-0.44	301.27	6	4	0
72.	Phyllemblin	1.23	198.17	5	3	0
73.	Physostigmine	1.94	275.35	5	1	0
74.	Pleurone	-0.47	114.06	4	0	0
75.	Protopine	2.75	353.37	6	0	0
76.	Pseudo hypercin	4.66	520.45	9	7	2
77.	Psilocybine	1.02	284.25	6	3	0
78.	Psoralen	2.29	186.17	3	0	0
79.	Quercetin	1.68	302.24	7	5	0
80.	Reserpine	5.01	60.69	11	1	3
81.	Schisandrin	3.71	418.49	7	2	0
82.	Schisantherin A	5.63	536.58	9	1	2
83.	Scopoletin	1.33	192.17	4	1	0
84.	Serpentine	-0.84	349.41	5	1	0
85.	Silibinin	1.47	482.44	10	5	0
86.	Silychristin	1.26	482.44	10	6	1
87.	Spathulenol	3.91	220.36	1	1	0
88.	Stigmasterol	7.87	412.70	1	1	1
89.	Sulforaphane	1.15	177.29	2	0	0

90.	Tabersonine	3.69	336.44	4	1	0
91.	Taraxasterol	8.10	426.73	1	1	1
92.	Theophylline	-0.01	180.17	6	1	0
93.	Tigloidine	2.41	223.32	3	0	0
94.	Triacontane	10.17	422.83	0	0	1
95.	Triterpenoid	3.22	552.77	7	3	1
96.	Tubocurarine	1.99	609.74	8	2	1
97.	Tymol	0.68	151.16	3	2	0
98.	Vinblastine	4.95	824.97	14	3	2
99.	Vincristine	4.95	824.97	14	3	2
100	Visnagin	2.30	230.22	4	0	0

ADMET parameter study-

Further studies related to ADMET were performed which helps to calculate several characteristics such as like Absorption, Distribution, Metabolism, Excretion and Toxicity of the compounds in the human system. The online server PreADMET is used for this parameter study (https://preadmet.bmdrc.kr/). The standard range of the above-mentioned aspects required for thecomputation of the pharmacokinetics parameters.

Table No.5- Standard Range of ADMET entities.

Parameters	Range	Prediction
Plasma protein binding (PPB)	More than 90% Less than 90%	Chemicals are strongly bonded Chemicals are weakly bonded
Blood Brain Barrier (BBB)	More than 1 Less than 1	Central nervous system active Central nervous system inactive
Human Intestine absorption (HIA)	0.0 ~ 20% 20-70%	Poorly absorbed Moderately absorbed

	70-100%	Well absorbed
Caco	Less than 4 4 ~ 70 More than 70	Low permeation Moderate permeation High permeation
Mutagenicity	No change in population Change in population	Negative mutagenicity Positive mutagenicity
Carcinogenicity	Negative Positive	Clear evidence of carcinogenicity No evidence of carcinogenicity

Table 6: ADMET analysis of compound

		Toxicity			Absorption				Distribution		Metabolism
S.No	**Compound**	**Mutagenicity[a] (Ames Test)**	**Carcinogenicity[b]**		**HIA%[c]**	**PCaco-d 2 (nm/sec)**	**PMDCK[e] (nm/sec)**	**PSkin[f] (nm/sec)**	**PPB%[g]**	**BBB[h] (CBrain/CBlood)**	**CYP2D6[i]**
			In Mouse	**In Rat**							
Reference/Standard Compounds											
1.	Donepezil	M	N	N	97.96	56.514	0.138	-3.041	84.615	O.187	Inhibitor
2.	Galantamine	M	N	N	95.402	20.930	78.091	-4.176	25.772	0.578	Inhibitor
Compounds											
1.	Agroclavine	M	N	N	97.108	43.722	110.676	-4.151	61.310	10.062	Inhibitor
2.	ajmaline,	M	N	N	93.200	22.817	41.071	-4.667	49.530	0.938	Inhibitor
3.	Alpha pinene	M	N	P	100	23.632	304.815	-1.446	100	5.533	NON
4.	Alpha-lapachone	M	NC	N	98.201	44.077	241.345	-2.882	83.583	2.003	NON
5.	Alpha-thujone	M	N	N	100.00	33.767	88.340	2.481	66.764	0.827	NON
6.	Alstonine	M	N	N	97.457	43.058	2.164	-4.435	53.818	0.477	NON
7.	Anthraquinone	M	N	P	99.177	21.988	60.456	-3.055	93.608	2.836	NON
8.	Apigenin	M	P	P	88.122	10.546	44.302	-4.145	97.253	0.565	NON

9.	Asarone	M	P	P	100.000	58.098	324.931	-1.680	93.393	1.229	NON
10.	Berberine	M	N	N	97.884	55.578	14.409	-4.373	58.542	0.693	Inhibitor
11.	Betaine	N	N	P	90.631	21.233	328.892	-3.981	67.594	0.154	NON
12.	Boscartin-C	N	P	N	92.152	18.931	198.647	-2.745	68.407	2.058	NON
13.	Bufotenine	M	N	N	89.469	5.660	85.161	-3.633	12.548	4.461	Inhibitor
14.	Butanol	M	N	N	99.72	15.06	55.29	-2.66	73.41	1.43	NON
15.	Butyric acid	M	N	N	86.76	20.17	74.40	-2.6	76.52	1.12	NON
16.	Cathine	M	N	N	91.036	18.8972	189.231	-3.093	000	0.515	Inhibitor
17.	Cimigenol	N	N	P	91.346	28.036	0.050	-3.996	92.214	2.467	NON
18.	Cineole	M	P	P	100.00	21.895	233.718	-1.321	100.00	1.467	NON
19.	Corynoxeine	M	N	P	96.083	23.081	7.977	-3.884	54.065	0.036	NON
20.	Coumarin	M	P	P	100.00	32.120	62.670	-2.043	43.395	1.568	NON
21.	Curcumin	N	N	P	94.403	20.073	99.989	-2.332	88.030	0.091	NON
22.	Daidzein	M	N	N	92.646	7.7203	31.422	-3.543	88.704	9.660	NON
23.	Epidherine	M	N	N	90.562	30.000	322.921	-2.65	6.879	1.536	Inhibitor
24.	Eugenol	M	P	P	96.774	46.886	342.148	-1.310	100.00	2.255	NON
25.	Galanthamine	M	N	N	95.402	20.930	78.091	-4.176	25.772	0.578	Inhibitor
26.	Gingerol	N	N	N	91.964	24.517	91.720	-1.639	100.000	1.474	NON
27.	Ginkgolide-B	N	N	P	40.304	20.572	0.612	-5.030	41.207	0.119	NON
28.	Harmaline	M	N	N	91.809	42.259	318.017	-4.369	62.089	4.536	NON
29.	Harmine	M	N	N	92.827	42.210	317.19	-4.386	64.050	3.798	NON
30.	Huperzine A	M	P	P	93.959	0.230	14.864	-3.705	55.669	0.462	NON

31.	HUPERZINE B	M	P	P	92.935	0.258	22.867	-4.373	67.247	0.677	NON
32.	Hydroxybenzyl isothiocyanate	M	N	N	95.986	21.303	54.490	-1.939	49.682	0.767	NON
33.	Hyoscine	M	P	N	95.828	25.717	4.613	-4.234	24.860	0.020	NON
34.	Isopimpinellin	M	N	N	98.31	47.6	170.7	-3.80	88.90	1.6	NON
35.	Kaempferol	M	N	P	79.439	9.577	29.611	-4.325	89.608	0.286	NON
36.	Limonene	M	N	P	100.00	23.631	238.434	-0.834	100.00	8.278	NON
37.	Linalool	M	N	N	100.00	29.355	115.421	-0.895	100.00	6.125	NON
38.	Linamarin	M	N	P	25.480	12.781	4.258	-5.165	15.988	0.287	NON
39.	Lochnerine	M	P	N	91.735	27.927	113.458	-4.786	50.448	3.725	Inhibitor
40.	Lycorine	M	P	N	92.419	21.624	6.383	-4.788	26.331	0.411	Inhibitor
41.	Mescaline	M	P	N	95.173	14.942	284.687	-2.491	27.829	0.799	Inhibitor
42.	Muscarine	M	N	N	95.948	46.214	315.528	-4.125	19.622	0.294	Inhibitor
43.	p - cymene	M	P	N	100	23.433	237.507	-0.805	100	4.969	NON
44.	Palmatine	M	N	N	97.923	55.956	1.207	-4.097	58.381	0.972	Inhibitor
45.	p-cymene	M	P	N	100.00	23.433	237.507	-0.805	100.00	4.969	NON
46.	Phyllemblin	M	N	N	72.040	0.188	26.380	-3.301	96.032	0.478	NON
47.	Physostigmine	N	N	N	95.072	27.953	46.654	-3.953	42.766	0.546	Inhibitor
48.	Pleurone	M	P	P	83.247	20.830	61.400	-2.743	65.909	0.645	NON
49.	Psilocybine	M	N	N	86.090	0.699	35.464	-2.776	60.148	0.612	Inhibitor
50.	Psoralen	M	P	P	83.247	20.830	61.400	-2.743	65.909	0.645	NON
51.	Quercetin	M	N	P	63.485	3.412	13.352	-4.433	93.236	0.172	NON

52.	Schisandrin	N	N	P	96.581	55.400	140.375	-2.910	85.456	0.043	NON
53.	Scopoletin	M	N	P	93.923	0.277	67.459	-3.135	29.418	0.644	NON
54.	Serpentine	M	N	N	94.064	39.182	5.547	-4.638	26.981	0.400	Inhibitor
55.	Silibinin	M	N	N	78.550	4.844	0.055	-4.236	87.754	0.060	NON
56.	Spathulenol	M	P	P	100.00	54.424	227.907	-1.329	82.888	6.966	NON
57.	Sulforaphane	M	N	N	97.883	5.591	11.165	-2.314	19.138	0.594	NON
58.	Tigloidine	M	P	N	100.000	55.696	55.187	-2.481	46.101	0.831	NON
59.	Tymol	M	N	N	100.000	38.012	87.330	-1.065	100.00	6.388	NON
60.	Visnagin	M	N	P	98.130	51.364	51.271	-3.735	80.505	2.078	NON

AutoDock

The docking was done with the help of AutoDock Tool 4.2.6 in order to find a suitable binding conformation of the target and ligand. The analysis of Binding conformation of the target-ligand complex was done and they were ranked according to the scoring function of the free energy of binding and inhibition constant (Cosconati S,2010). Four coordinate files are created ligand.pdbqt, receptor.pdbqt, grid.gpf and dock.dpf.

RESULTS AND DISCUSSION

Pharmacological studies:

The process of identification of compounds from chemical libraries that are “drug-Like” is usually preferred in the area of computer-aided drug designing. The most preferred method of drug-likeliness analysis is the Lipinski’s rule of 5. The compound binds efficiently to the receptor if the violation is 1 or 0. The compounds that have the violation number more than 2, are rejected from further screening procedure. Physiochemical properties like Drug-likeness and ADMET were studied of all the 100 compounds obtained from available literature and NPACT database. The parameters such as molecular weight, Hydrogen bond donor, Hydrogen bond acceptor, XlogP3 and N-violations were studied. If the N-violation is 0, it assumes that compound efficiently binds to the target.

Autodock

Docking study was done to understand the basis of structure of protein-ligand interactions. Docking studies were conducted on all the five compounds and the standard drug donepezil and galantamine against the target protein. The compounds were further analysed on the basis of binding energy and inhibition constant.

Table number 7 contains the results obtained after performing the AutoDock experiment. The docking outcomes revealed two promising and potential inhibitor against protein target AcHE (PDB:4EY7) . The result obtained showed that Cyclosativane gives the lowest free energy of -5.52 kcal/mol, -3.99kcal/mol, -3.07 kcal/mol, -8.32 kcal/mol and -7.37 kcal/mol and low inhibition constant in complex with Asiatic acid, Butyric acid,2-Butanol,Daidzein and Limonin respectively. Daidzein is giving better score than the two standards-Donepezil and Galantamine. Further, the compoundalso satisfies the Lipinski’s rule and ADMET

Table 7: Obtained Docking Score of the Compounds.

S.No.	Compounds	4EY7
Reference/Standard Compounds		
1.	Donepezil	-7.8kcal/mol 610.77mM
2.	Galantamine	-8.2kcal/mol 608.10mM
Compounds		
1.	Alpha- lapachone	-7.86kcal/mol 1.73uM
2.	Harmaline	-6.62kcal/mol 14.14uM
3.	Harmine	-6.81kcal/mol 10.24uM
4.	Daidzein	-8.61kcal/mol 489.42uM
5.	Isopimpinellin	-6.71kcal/mol 12.05uM

The 3D structure of the complex-

The ligand and protein interaction site wasanalyzed for its basic chemical properties. Our standard Donepezil and Galantamine ligand-protein complex's binding site shows to be surrounded by amino acids-PRO266,ASP344,GLN444,PRO441,HIS436,TRP563 and PRO568 for Galantamine YR103,TYR155,TRP317,SER324,and TYR372 Similarly, the binding pocket of Daidzein complex also shows the presence of somewhat similar amino residues. Amino acids that are surrounding our two best ligand complexes (Daidzein) are PHE297,TYR124,TYR337,PHE338,TRP286,PHE295,VAL294,ARG296,SER293,TRY341and LEU289(Figure 8,9,10 and 11). The amino acids involve in the protein and ligand complex formation both in our two standard complexes and best ligand complexhave similarity in the presence of residue.

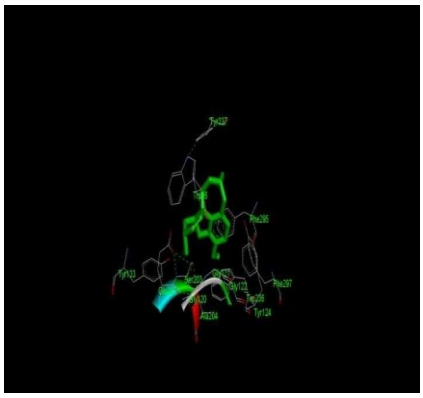
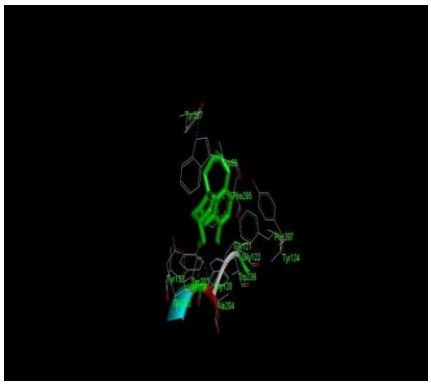


Figure9: Donepezil



igure10: Galantamine

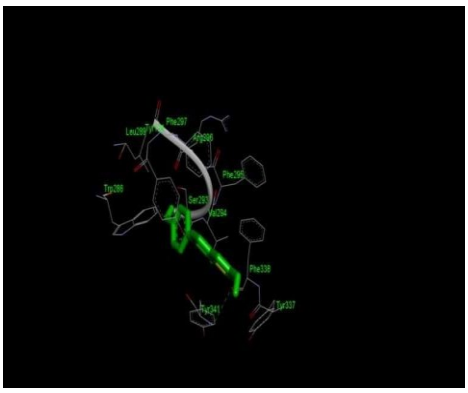


Figure11: Daidzein

DISCUSSION

In the present study, *in-silico* approach was applied on five compounds that were retrieved from various research papers and PubChem site against 4EY7 of Acetylcholinesterase that wereretrieved from RCSB PDB site. The approach uses the process of fitting molecules computationally in the target protein by utilizing the tertiary structures of the active site and then eventually ranking all the 5 compounds on the basis of their interaction profile. However, the identification and development of novel effective drugs is a lengthy and costly affair. It almost takes 8-12 years, including trials and regulatory approval. Focusing on this and the already reported plant derived secondary metabolites and main component in mind, the present in silico study is designed. Among the studies compounds, Daidzein came out as the most efficient and promising compounds against the targets selected. Based on outcomes of drug-likeness, ADMET and docking, we hypothesized that the compiled compound particularly, Daidzein showing higheraffinity and stability for the selected target.

CONCLUSION

Compounds obtained from natural source have an important part in the treatment and prevention of human diseases. Our in-silico study offers a clear idea that compounds are plant derived secondary metabolites can be highly potent in inhibition of the enzyme responsible for the progression of the deadly Alzheimer's disease. In silico studies and Molecular docking are the best methods to select potential candidates for making a drug. The purpose of this study was to identify compounds from natural source that can inhibit the enzyme called acetylcholinesterase (PDB;4EY7). Over all one hundred compounds that are compiled from available literature, NPACT database and studied by in-silico approach. Our outcomes were supported by analyses such as the estimation of free energy. The results obtained showed that Daidzein is the most potential compound that can be used against Alzheimer's disease offering better energy score as compared to the drugs that are clinically tested. This compound has also satisfied the Lipinski rule of five and ADMET prediction. the compound has no violations. In conclusion, the chief compound revealed that it has the potential to treatAlzheimer's disease patients. Therefore, this best candidates for Alzheimer's disease have to be taken up for further analysis. These outcomes encourage the further in vitro and in vivo studies.

Hence, we believe that the following compound can be efficient anti-Alzheimer drugs; therefore, more research should be conducted in this field to verify its potential.

Author Contribution Mohd Aziz & Firdaus Jahan done the compilation of various molecules from the literature. After compilation of the molecule Mohd Aziz done the lipinski analysis for further more studies for screening ADME analysis is done by all the three members respectively. Docking and result analysis and reading done by the Ms. Firdaus Jahan . Manuscript and review of literature work done by Dr. Kavita and she wrote the most of manuscript with concern of Mohd Aziz & Firdaus Jahan. The manuscript was written and reviewed by all authors. All authors read the manuscript and approved the submission.

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Data Availability available from the corresponding author upon request.

Declarations

It's a *insilico* computational study so no need of ethical approval because *insilico* study not needany patient and animal.

Informed Consent It is computational study all study done by using computation tools so noneed of any consent because we not use any human and animal as a sample.

Conflict of Interest The authors declare no competing interests.

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