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in silico Single Nucleotide Polymorphism Prediction and Design for Targeting Amyloid Precursor Protein in Alzheimers Disease

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Alzheimer's disease is a progressive neurodegenerative disorder, is the common cause of dementia and affect life quality. Present research analysis is based on the amyloid precursor protein as it is one of the major biomarkers of Alzheimer's disease, the insoluble part of which gets deposited due to the inappropriate cleavage by secretase enzyme. The instrumental part of this study was performed by using Computer Aided Drug Design (CADD). About 23 phytoconstituents were taken, from these constituents rosamarinic acid produced the highest docking score. Preliminary characterizations of all the ligands were conducted using Biovia Discovery studio along with protein characterization, Lipinski rule analysis and ADMET. The *in silico* analysis convincingly predicted the action of rosamarinic acid, against the formation of amyloid plaque by binding with the amyloid precursor protein .Details of single nucleotide polymorphism on gene APPI were analyzed by dbSNP database and displays the need for further *in vitro* study.

Keywords: Alzheimer, Amyloid protein, Biovia, docking, Secretase Enzyme, ProtParam.

INTRODUCTION

Alzheimer's disease is one among the neurodegenerative disorder, where the main setback is the loss of neurons in the hippocampus and basal fore brain. Among all the dementia cases reported Alzheimer's disease account for almost 60 % [1]. According to world report on Alzheimer's disease, it is found that 35.6 million people suffer from this neurodegenerative disorder in 2010 and going on escalating to 67.57 million by 2030 [2].

It is mainly associated with brain shrinkage. They occur mainly at the age of 65 and above and then progress with age. Family history of dementia only plays a petite role. The two main marked microscopic features are extracellular amyloid plaques and intraneuronal neurofibrillary tangles [3-6]. Amyloid plaque is characterized by deposits of β-amyloid plaques and other with phosphorylated form of microtubule associated protein [7,8]. The erroneously processed amyloid peptide is the major contributory factor of this disease [6]. In normal circumstances amyloid precursor protein is being cleaved by α-secretase into two parts, one which is the large soluble portion and the other being small membrane anchored part which is then cleaved by γ-secretase [9]. There is also an additional route of cleavage of amyloid precursor protein which is by β-secretase to produce the soluble fragment. But the problem arises when this is being cleaved by γ-secretase instead of β-secretase to produce less soluble amyloid β-peptides. The amyloid β-peptides now get to aggregate to produce the main character of Alzheimer's disease which is amyloidfibrils. This happens as the γ-secretase lacks precision and cut with varying lengths to form A β-40 and A β-42. Endosomal compartment witness the cleavage by β-secretase then α-secretase pathway, while proteolysis by α-secretase takes place in cell membrane. But there is still no evidence to suggest that Alzheimer's disease patients are dominated by β-secretase pathway. But the case is more likely be occurring due to the impaired clearance of fibrillogenic amyloid β-peptides [10]. The amyloid deposits are present in diseases other than Alzheimer's diseases which include Parkinsonism, senile systemic amyloidosis and even in type 2 diabetes mellitus [11].

Curcumin from *Curcuma longa* belonging to the ginger family was being used for centuries as a herbal medicine [12].

It is found to have a promising effect on dementia therapy due its effectiveness as a antioxidant, anti-amyloidogenic and anti-inflammatory actions [13,14]. Epigallocatechin gallate which is a flavanol obtained from *Camellia sinensis* due to its trihydro group in its B ring and the esterified gallate moiety in the C ring contribute to its antioxidant action [15,16]. Asiatic acid from *Centella asiatica* which is a triterpine have antioxidant and neuroprotective action. It helps in preventing mitochondrial membrane dysfunction and apoptosis [17]. Vincristine and vinblastine have antioxidant and also it arrest the cell division at metaphase and thus acting as an anticancer agent [18]. *Withana somnifera* contains withanolides and withaferin which helps in learning and memory improvement in Alzheimer's disease and they also affect the plaque pathology and reduces the load due to amyloid deposites [19]. Quercetin a flavanol helps in counteracting oxidative stress [20]. Linalool acts by reducing the histophathological hallmarks of Alzheimer's disease by affecting the inflammatory mediators and non-amyloidogenesis [21,22]. Kaempferol is found to have protective action on impaired performance due to amyloid peptides and oxidative stress inducing cell death [23]. Other phytoconstituents like butylated hydroxytoluene, myricetin, myristicin are also found to have antioxidant action [24]. The phytoconstituent rosamarinic acid belonging to the family laminacea is found to have antioxidant activity and inhibition of acetyl and butyryl cholinesterase that helps in the symptomatic relief in Alzheimer's disease. Now in this *in silico* studies prove that the rosamarinic aid is found to have inhibitory action on amyloid precursor protein [25]. Epigallocatechingallate is found to reduce the amyloid β-protein by cleaving the APP in the non-amyloidogenic pathway through the α-secretase to produce soluble amyloid protein. It is found to have neuroprotective effect in the hippocampal pathway in case of neuronal damage with their action as ischemic induced increase in putrescine level [26-28]. In this study, the standard compound chosen are zinc and Congo red. Zinc is one of the essential trace element in the human biology and found to prevent the amyloid plaque generation in the brain cells. It is also being reported that the zinc reversibly block the amyloid poly-peptide. It is being previously reported [29] that amyloid plaques is able to form ion channel in the lipid layer and cause in the cell destruction. Thus study is made on the action of Congo red on the inhibition of this ion channel.

EXPERIMENTAL

Curcumin, kaempferol, anaferine, myricetin, butylated hydroxytoluene, rosmarinic acid, epigallocatechin, myristicin, linalool, withanolide A, withaferin A, ascorbic acid, withanolide E, withanolide D, β-sitosterol, quercetin, asiatic acid, campestrol, madecassic acid, vinblastine, vincristine, catharanthine and α-pinene were the ligands used for the study. The phyto-constituent constituents with their IUPAC nomenclature are tabulated as Table-1. The information about the ligands, their 3D structure and canonomical smiles were collected from Pubchem, a chemical database and these were saved in PDB format. The 3D structure of phytoconstituents like kaempferol, curcumin, epigallocatechingallate, rosamarinic acid, myricetin that showed good binding energy is shown in Fig. 1.

The Biovia Discovery studio Version 2017 v 17.2.0.16349 was used for the characterization and depiction of ligands with its ADMET studies and the ADMET plot is drawn. Lipinski rule of five devised was used to subject the ligands for drug likeness for determining their structural and molecular properties. The rule formulated under Lipinski laid the following conditions for a ligand to be satisfactorily considered as a drug which include molecular weight to be less than 500 Da, lipophilicity not greater than 5, hydrogen bond donors not less than 5, hydrogen bond acceptors not less than 5 [30-32]. Among the whole ligands taken about 90 % ligands obey the criteria of Lipinski rule to be fulfilled as a drug. Amyloid precursor protein was selected as the target protein as being one of the main characteristic features of Alzheimer's disease in regions where memory and cognition are important. The structure of protein was collected from the RCSB protein data bank and the protein secondary characterization was done using secondary structure prediction method with alignment (SOPMA) which displays α- and π- helix random coil [33]. Further then use ProtParam- a tool to analyze the primary structure [34,35] and which includes the characterization using chemical and physical properties like aliphatic index, instability index, half life,

TABLE-1
IUPAC NAME OF PHYTOCONSTITUENTS WITH ANTI ALZHEIMERS ACTIVITY

Chemical constituents	IUPAC nomenclature
Curcumin	(1E,6E)-1,7-*bis*(4-Hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
Kaempferol	3,5,7-Trihydroxy-2-(4-hydroxyphenyl)chromen-4-one
Anaferin	1,3-*bis*[(2R)-Piperidin-2-yl]propan-2-one
Butylated hydroxytoluene	2,6-di-*tert*-Butyl-4-methylphenol
Myricetin	3,5,7-Trihydroxy-2-(3,4,5-trihydroxyphenyl)chromen-4-one
Roamarinic acid	(2R)-3-(3,4-Dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxypropanoic acid
Epigallocatechin	[(2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2*H*-chromen-3-yl]-3,4,5-trihydroxybenzoate
Linalool	3,7-Dimethylocta-1,6-dien-3-ol
Myristicin	3,5,7-Trihydroxy-2-(3,4,5-trihydroxyphenyl)chromen-4-one
Withanolide A	(22R)-6α,7α-Epoxy-5,20,22-trihydroxy-1-oxo-5α-ergosta-2,24-dien-26-oic acid δ-lactone
Withaferin A	4β,5β,6β,22R)-5,6-Epoxy-4,22,27-trihydroxy-1-oxoergosta-2,24-dien-26-oic acid δ-lactone
Ascorbic acid	(2R)-2-[(1S)-1,2-Dihydroxyethyl]-3,4-dihydroxy-2*H*-furan-5-one
Withanolide E	(5β,6β,17α,22R)-14,17,20-Trihydroxy-5,6:22,26-diepoxyergosta-2,24-diene-1,26-dione
Withanolide D	5,6-Epoxy-4,20,22-trihydroxy-1-oxoergosta-2,24-dien-26-oic acid δ-lactone
β-Sitosterol	(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5-Ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol

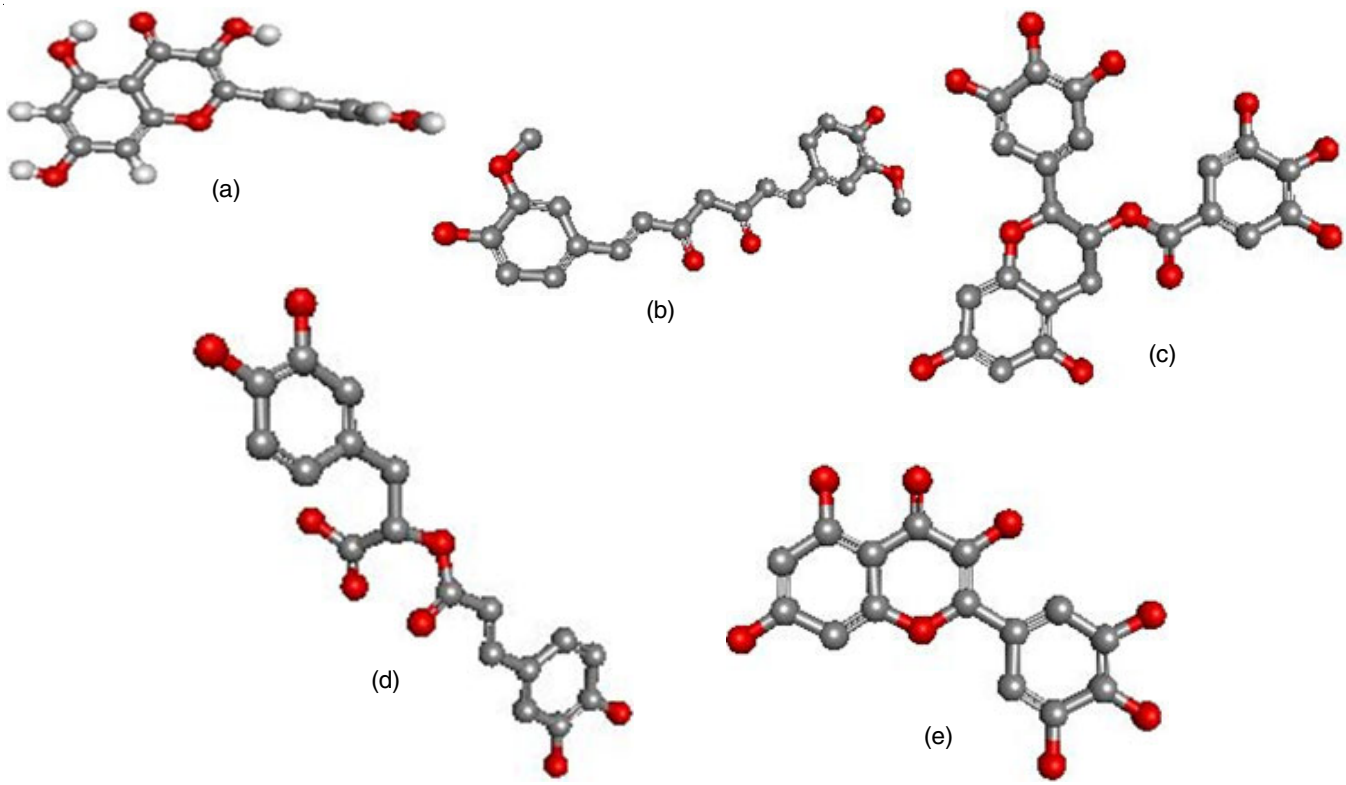

Fig. 1. Ball and stick representation of kaempferol (a), curcumin (b), epigallocaechingallate (c), rosamarinic acid (d) and myrsticin (e)

molecular weight and theoretical Pi. A protein is considered to be best when they satisfy high half life, low instability value with low α-helix and moderately high β-turn. The proteins taken were 2LOH, 2LZ3 and 2LZ4 were subjected to primary and secondary characterization. The protein 2LOH with high half life, low instability value was selected for the study. The docking interactions protein was then subjected to cleaning procedure to remove the cells, water molecules and any hetero atoms and followed with docking procedures. Details of single nucleotide polymorphisms is obtained from online database dbSNP short genetic variations. Deleterious amino acid substitution prediction is done by SIFT tool [36-41] and POLYPHEN-2 (polymorphism phenotyping v2 is a online tool narrates effects of amino acid substitution on the structure and function of protein) [42-44].

RESULTS AND DISCUSSION

Apart from the docking studies the preliminary characterization of all the ligands were also carried out using Biovia Discovery studio Version 2017 v 17.2.0.16349. Quality of ligands were calculated using ADMET studies and where successfully produced in Table-2.

Five constituents were found to cross the blood brain barrier *viz*., anaferin, butylated hydroxytoluene, rosamarinic acid, linalool and epigallocatechingallate. The study shows that all the ligands were found to be non-carcinogen and non-toxic in the AMES toxicity studies. Their ADMET plot is also produced in Fig. 2.

In the Lipinski rule characterization, it was found that β-sitosterol, medecassic acid and vincristine desecrated the law with molecular weight greater than 500 Dalton and in case of TPSA parameter, epigallocatechin gallate and vincristine

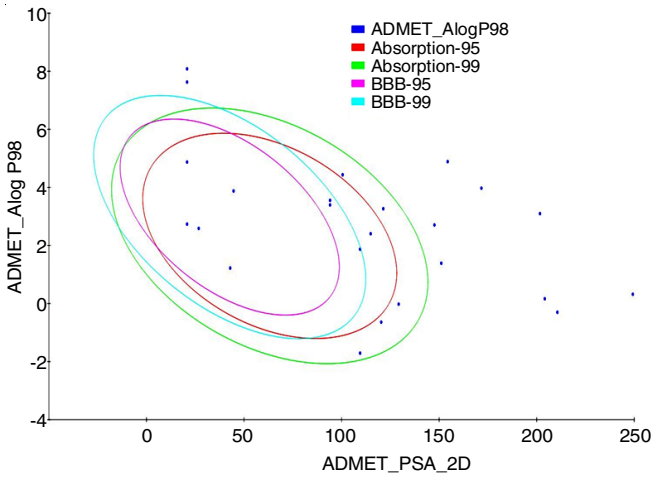

Fig. 2. ADMET plot of 23 ligands

disobey it with TPSA value greater than 160. Along with this β-sitosterol have a lipophilicity value greater than 5 thus violating the drug likeliness (Table-3).

3D structure of both ligand and protein where displayed using Biovia for docking course of action to be carried out with high efficiency. The opening studies for the research study about the ligands and targets where conducted. Initially three protein with ID No. 2LOH, 2LZ3, 2LZ4 were taken. From these 3, the protein for the docking study was selected based on their characters using Protparam (Table-4) and SOPMA tools (Table-5).

The favoured one was 2LOH as it possess low α-helix and high random coil and further having long half life of 30 h. In addition, it was very much stable than the other two. Twenty four ligands where made to dock with three sites in the protein.

TABLE-2
ADMET STUDIES OF THE PHYTOCONSTITUENTS AND STANDARD DRUG

Ligands	BBB	HIA	CYP Inhibitor				AMES toxicity	Carcinogenicity
			CYP1A2	CYP2C9	CYP2D6	CYP3A4		
Curcumas	0.6162	0.9539	Inhibit	Inhibit	Inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Kaempferol	0.628	0.98	Inhibit	Inhibit	Non-inhibit	Inhibit	Non-toxic	Non-carcinogen
Anaferin	0.941	0.9234	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Butylated hydroxytoluene	0.95	0.9941	Inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Myricetin	0.5711	0.965	Inhibit	Non-inhibit	Non-inhibit	Inhibit	Non-toxic	Non-carcinogen
Roamarinic acid	0.577	0.965	Inhibit	Non-inhibit	Non-inhibit	Inhibit	Non-toxic	Non-carcinogen
Epigallocatechingallate	0.60	0.88	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Linalool	0.99	0.97	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Myristicin	0.9504	0.994	Inhibit	Inhibit	Inhibit	Inhibit	Non-toxic	Non-carcinogen
Withanolide A	8.327	0.895	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Withaferin A	0.869	0.808	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Ascorbic acid	0.8532	0.6559	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Withanolide E	0.610	0.618	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Withanolide D	0.7979	0.8736	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
β-Sitosterol	0.694	0.878	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Quercetin	0.577	0.96	Inhibit	Non-inhibit	Non-inhibit	Inhibit	Non-toxic	Non-carcinogen
Asiatic acid	0.74	0.94	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Campesterol	0.97	1	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Madecassic acid	0.74	0.94	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Vincristine	0.95	0.97	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Vinblastine	0.9203	0.9806	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Catheranthine	0.9452	0.9918	Non-inhibit	Non-inhibit	Non-inhibit	Inhibit	Inhibit	Non-carcinogen
α-Pinene	0.89	0.996	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Non-carcinogen
Zinc	0.9733	0.9838	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Non-toxic	Carcinogen
Congo red	0.753	0.921	Non-inhibit	Non-inhibit	Non-inhibit	Non-inhibit	Toxic	Carcinogen

BBB = Blood brain barrier; HIA = Human intestinal absorption

TABLE-3
EVALUATION OF THE DRUG LIKENESS OF THE HERBAL CONSTITUENTS

S. No.	Chemical constituents	miLog P	TPSA	natoms	m.w.	nOH	nOHNH	nrotb	Volume
1	Curcumin	2.30	93.07	27	36.38	6	2	8	332.18
2	Kaempferol	2.17	111.12	21	286.14	6	4	1	232.07
3	Anaferin	1.38	41.12	16	224.34	3	2	4	236.41
4	Butylated hydroxytoluene	5.43	20.23	16	220.36	1	1	2	241.00
5	Myricetin	1.39	151.58	23	318.24	8	6	1	248.10
6	Roamarinic acid	1.63	144.32	26	360.32	8	5	7	303.54
7	Epigallocatechin	2.25	197.36	33	458.38	11	8	4	367.57
8	Linalool	2.43	20.23	10	140.23	1	1	4	159.03
9	Myristicin	2.44	27.7	14	192.21	3	0	3	178.05
10	Withanolide A	4.15	96.36	34	470.61	6	2	2	441.81
11	Withaferin A	3.86	6.36	34	470.61	6	2	3	442.38
12	Ascorbic acid	-1.40	107.22	12	176.12	6	4	2	13.71
13	Withanolide E	3.18	116.59	35	486.71	7	3	2	449.16
14	Withanolide D	4.15	96.36	34	470.61	6	2	2	441.84
15	Beta sitosterol	7.15	99.38	41	576.86	6	4	9	588.64
16	Quercetin	1.68	131.35	22	302.24	7	5	1	240.08
17	Asiatic acid	4.70	97.98	35	488.71	5	4	2	487.79
18	Campesterol	8.30	20.23	29	400.69	1	1	5	439.70
19	Madecassic acid	3.78	118.21	36	504.21	6	5	2	495.83
20	Vincristine	4.95	171.18	60	824.97	14	3	10	747.07
21	Vinblastine	5.56	154.11	59	810.99	13	3	3	744.65
22	Catharanthine	3.99	45.33	25	336.44	4	1	3	315.99
Standard drug									
1	Zinc	-0.39	0	1	65.39	0	0	0	40.59
2	Congo red	3.90	215.09	46	650	12	4	7	520.25

TPSA: Total polar surface area, m.w.: Molecular weight, nON: Number of hydrogen bond acceptors, natoms: Number of atoms, nOHNH: Number of hydrogen bond donors, nrotb: Number of rotatable bonds.

TABLE-4
SECONDARY CHARACTERIZATION OF PROTEIN USING SOPMA

S. No.	PDB ID	Alpha helix	Pi helix	Random coil	β-Turn
1	2LZ3	21	0	2	10
2	2LZ4	31	0	3	9
3	2LOH	8	0	18	12

TABLE-5
PRIMARY CHARACTERIZATION OF PROTEIN USING ProtParam

S. No.	PDB ID	No of amino acids	Theoretical pi	m.w.	Instability index	Aliphatic index	Half life
1	2LZ3	62	10.85	6260.17	4.11	169.68	4.4
2	2LZ4	62	10.5	6324.29	4.11	160.32	4.4
3	2LOH	86	10	8872.93	12.43	142.56	30

About 7 constituents showed the required action with an increased docking energy than the standard one. This is represented in a graph. The docking analysis was tabulated with their different docking scores at the three sites and specified in Table-6.

And it was finalized to be rosamarinic acid with the highest CDocker value and with all needed properties. This was then compared with the standard on zinc and Congo red and found to have higher value. In addition, in the preliminary screening of the standard one Congo red and zinc was found to be carcinogen. The docking of protein 2LOH with rosamarinic acid is shown in Fig. 3 and their chemical interaction with various amino acid involved is visualized in Fig. 4. Finally, a graph was plotted with the docking score at the three sites as shown in Fig. 5.

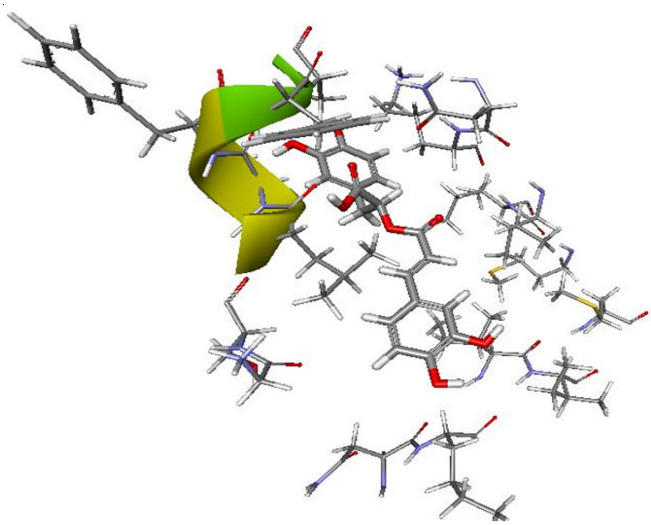

Fig. 3. Docking of protein with PDB ID: 2LOH with rosamarinic acid

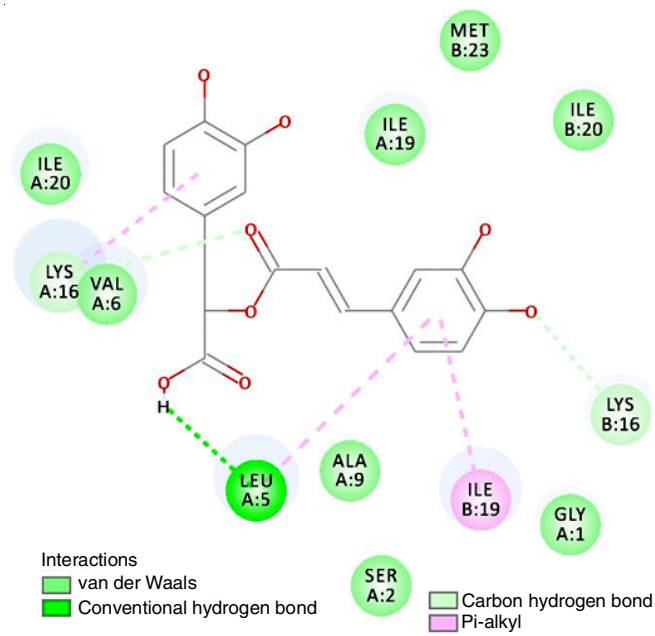

Fig. 4. Docking with the interactions and amino acid involved

Mutations in critical regions of amyloid precursor protein, including the region that generates amyloid beta (Aβ), cause familial susceptibility to Alzheimer's disease [45-47]. Single nucleotide polymorphisms associated with amyloid precursor protein gene APPI was found to be 71529. From this total SNPs, 486 covers the missense mutation and 241 count to be coding synonymous. 5'utr and 3'utr region containing 188 and 334 SNPs, respectively. Nonsense SNPs are 10 and frame shift SNPs are 9. From polyphen data, found that only 2 rs id cause probably damaging are 1800557 and 5588932. Cause of

TABLE-6
DOCKING SCORE AT THREE DIFFERENT SITES

Chemical constituents	Site 1		Site 2		Site 3	
	-CD Docker energy (kcal/mol)	-CD interaction energy	-CD Docker energy (kcal/mol)	-CD interaction energy	-CD Docker energy (kcal/mol)	-CD interaction energy
Kaempferol	20.52	25.42	25	31	16.2	21
Curcumn	18.58	28.24	24	33.08	19	28
Epigallocatechingallate	25.81	27.35	25.9	27.2	27.3	28.8
Myricetin	19.9	20.41	23	25	20.9	21.5
Anaferin	6.04	22.37	–	–	–	–
Butylated hydroxytoluene	4.9	16.25	–	–	–	–
Rosamarinic acid	–	–	32	32	29.4	32

TABLE-7
SINGLE NUCLEOTIDE POLYMORPHISM, SIFT PREDICTION

SNP	Amino acid change	SIFT score	SIFT median	No. of SEQS at position	SIFT prediction
rs63750264	V717F	0.001	2.48	101	Deleterious
rs63750264	V642F	0.001	2.43	101	Deleterious
rs1800557	A713V	0.001	2.48	100	Deleterious
rs1800557	A695V	0.001	2.52	100	Deleterious
rs63750579	E674Q	0.022	2.59	87	Deleterious
rs63750579	E669Q	0.022	2.63	88	Deleterious
rs63749964	V717G	0	2.48	101	Deleterious
rs63749964	V642G	0	2.43	101	Deleterious
rs63750066	A638T	0.002	2.43	100	Deleterious

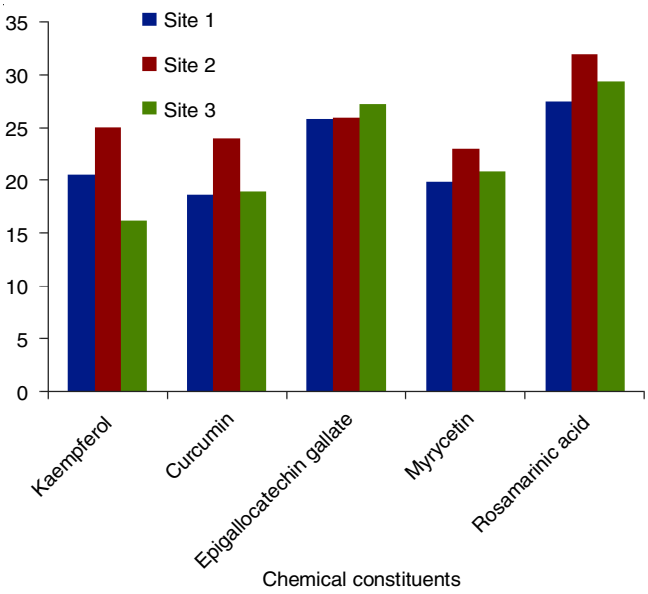

Fig. 5. Graph of docking score of phytoconstituents at 3 different sites

damaging is due to change in amino acid at a particular position. At 713 position alanine change to lysine cause rs id 1800557 leads to probably damaging and glutamic acid change to lysine at 501 position leads to probably damaging of rs id 5588932.

From the SIFT online tool, SIFT score ranges from 0 to 1. The amino acid substitution is predicted damaging is the score is <= 0.05, and tolerated if the score is > 0.05. Sift median ranges from 0 to 4.32, ideally the number would be between 2.75 and 3.5. This is used to measure the diversity of sequences used for prediction. A warning will occur if this is greater than 3.25 because this indicates that the prediction was based on closely related sequences. Sequence at position is the number of sequences that have an amino acid at the position of prediction. Substitution at position 717 from valine to phenylalanine predicted to be deleterious. Result of SIFT online tool details are specified in Table-7.

Conclusion

The CADD analysis was very helpful in predicting the ideal characteristics of both the protein and the ligands and aided in highlighting the useful phytoconstituents for diseases. All the conventional therapies for Alzheimer's disease produce only symptomatic relief and neither of them affects the amyloid plaque formation thus this study is a major break through for this treatment. From the *in silico* analysis, it was concluded that rosamarinic acid was useful to act against amyloid precursor protein to prevent its redundant cleavage by the secretase enzyme into amyloid plaque further assisting in the neuroprotective effect in Alzheimer's disease. Additionally this compound obeys all the rules to show the drug likeliness behaviour under the Lipinski rule. The phytoconstituent also shows more drug docking score than the standard compounds zinc and Congo red with the same mechanism of action. Now, it sets a new path to find out through the *in vitro* studies in future.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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