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Targeting BACE 1(Beta secretase) through Polyphenolic compounds -A computational *insilico* approach with emphasis on binding site analysis

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ABSTRACT

Polyphenolic compounds posses vast number of biological activities and they are the inclusions of phyto-constituents of plant kingdom. Synthetic drugs used in the treatment of neurodegenerative disorders like Alzheimer's disease is of only symptomatic and for not permanent cure over the progression of the disease. Beta secretase-1(BACE1) is aspartic protease makes improper cleavage of amyloid precursor protein (APP) found on the membrane of the neuronal cells and produces the accumulation of beta amyloid proteins of insoluble fractions. Polyphenolic compounds like catechin3gallate, hesperidin, hesperitin etc., were found to possess lowest binding energy with best conformation, when comparing with the standard reference ligands. In this ***insilico docking studies,*** *it revealed that targeting BACE1 inhibition, through Polyphenolic compounds can create number of lead molecules for better therapeutic concern in future.*

Key words: Polyphenolic compounds, Beta secretase, Alzheimer's disease, MVD 6.O

INTRODUCTION

Computational methodologies and their tools made drug discovery process less time consuming and decreased the usage of animals in prior to preclinical studies. *In silico* studies were employed for the simulation of physiological systems including physiological macromolecules like receptors, enzymes were designed using modeling softwares and analysed for their simulating activities. In this advanced drug discovery process the simulated proteins can be targeted, if any underlying implications are because of all those enzymes.

Naturally occurring phyto-compounds, such as polyphenolic antioxidants found in fruits, vegetables, herbs and nuts, may potentially hinder neurodegeneration, and improve memory and cognitive function **[1]**. Neurodegenerative disorders like Alzheimer's disease (AD) is thought to be caused by the progressive brain accumulation of β-amyloid (Ab) peptides into fibrillar aggregates and insoluble plaques resulting severe memory loss and neuronal cell death **[2]**. AD develops gradually and induces memory loss, unusual behavior, personality changes, and a general decline in thinking abilities and it affects people above the age of 60. A non peptide, optimum molecular weight, potent BACE1 inhibitors development is of major importance **[3]**.In such a way the non-peptidic inhibitors are of increasing importance in order to cross the blood brain barrier and after crossing BBB it to be escaped from

metabolic enzymes before and after reaching the neuronal cells. The prevalence of AD is of more in males than in females. Therapeutic targets is of symptomatic and they are AChE inhibitors and thereby formation of new memories **[4]**. At molecular level, pathogenesis of AD has various stages of development. The symptoms of AD are possible only after a 75% degradation of CNS neurons involved in the memory formation. Moreover acetylcholine receptors will be degraded, so that the remaining Ach can be made available to little cholinergic receptors for their binding and thereby storage of memory through cellular processing. As in the genesis of AD, an enzyme responsible for degrading the acetylcholine at the vicinity of receptor is of AChE. At present, therapeutic AChE inhibitors are of major concern in AD patients. Research has created to focus on various cellular events and their elements to be targeted which are responsible for the development of AD **[5]**. The evidence is based on the genetic observations from familial Alzheimer's disease. This research showed that mutation of the genes of amyloid precursor protein , presenilin-1 and presenilin-2 that cause inherited Alzheimer's disease lead to increased accumulation of fibrillary β-amyloid in the brain.**[6]** Accumulation of beta amyloid plaques and neurofibrillary tangles are the hallmarks of atrophied brain tissues in AD patients. Beta secretase *(*BACE1*)* protein a membrane-bound aspartic protease, cleaves *beta*-amyloid precursor. In normal condition BACE1 cleaves and produces a soluble beta amyloid in the plasma of neuronal cells.**[7]** But due to various etiological processes the abnormal breakdown of beta amyloid precursor by BACE-1, it forms a insoluble beta amyloid plaques. These formed beta amyloid particles aggregate together and forms beta amyloid plaques. Targeting beta secretase is of a difficult task as a therapeutic concern, but the access towards it has to be done with developing a drug moiety with more affinity and penetration towards the enzyme. Targeting the enzyme (BACE1) with peptides is problematic due to the risk of degradation by the enzymes. **[8]**

MATERIALS AND METHODS

Enzyme under investigation, the crystal structure of β- secretase (BACE1) with PDB code -3H0B, was downloaded from the RCSB protein data bank. Latest version of Mole Gro virtual docker (MVD) 6.0 was downloaded from www.**clc**bio.com; Accelerys Discovery studio visualizer 3.1–downloaded from www.accelerys.com, Chem Office package- Chem 3D ultra- from www.cambridgesoft.com.

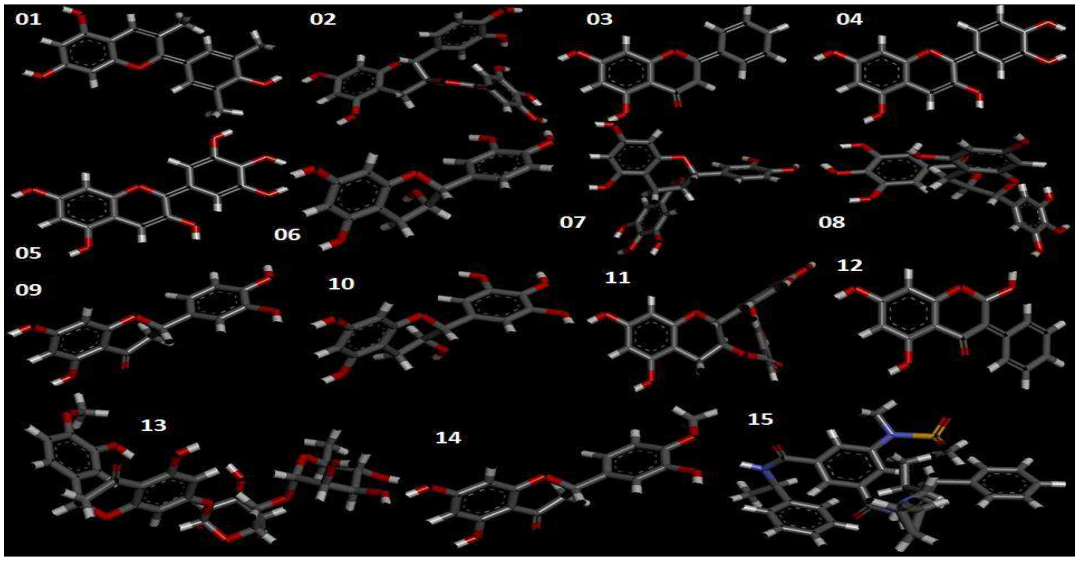

Fig 1: Optimized ligands for docking process 1.Anthocyanidin 2. Catechin-3-gallate 3. Chrysin4. Cyanidin 5. Delphinidin 6.Epicatechin 7. Epicatechin -3-gallate 8. Epigallocatechin-3-gallate 9.Eriodictyl 10. Gallocatechin 11.Gallocatechin -3-gallate 12.Genistein 13.hesperidin14.hesperitin 15. Beta secretase inhibitor-IV (Calbiochem)

The 3 dimensional structures of the ligands (Anthocyanidin, Catechin3gallate, Chrysin, Cyanidin, Delphinidin, Epicatechin, Epicatechin3gallate, Eriodictyl, Gallocatechin, Gallocatechin-3- gallate, Genistein, Hesperidin, Hesperitin) are obtained by designing them in ChemDraw ultra 9.0 and the energy of the ligand was minimized for

lead optimization using chem3D ultra 9.0 which runs MOPAC energy minimization job and 3D structures obtained after MOPAC job are visualized through accelerys discovery studio 3.1.

METHOD

The enzyme molecule is refined by using Accelerys Discovery studio 3.1. ***In silico*** docking study was performed using commercial **Mole Gro virtual docker 6.0**. The ligands were designed using chem draw ultra and their energy is minimized (ligand optimization) using ChemDraw 3d ultra which runs MOPAC job for energy minimization. The fig.1 shows the optimized ligands used for the study. MolDock is based on a new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm. Extension of the piecewise linear potential (PLP) including new hydrogen bonding and electrostatic terms is the scoring function used in Mol Dock . a re-ranking scoring function is used in order to improve the docking accuracy, which identifies the most promising docking solution from the solutions obtained by the docking algorithm. The MolDock scoring function (MolDock Score) is derived from the PLP scoring functions **[9, 10]** and later extended **[11]**. The Mol Dock scoring function further improves these scoring functions with a new hydrogen bonding term and new charge schemes. The docking scoring function, Escore, is defined by the following energy terms:

$$E_{score}=E_{inter}+E_{intra} \qquad \text{…….. (1)}$$

Where E_{inter} is the ligand –protein interaction energy

$$E_{\text{intra}} = \sum_{i\in \text{ligand}} \sum_{j\in \text{ligand}} E_{\text{PLP}}(r_{ij}) + \sum_{\text{flexible bonds}} A[1-\cos(m\cdot\theta-\theta_0)] + E_{\text{clash}} \qquad \text{…… (2)}$$

Mol Dock is based on a new hybrid search algorithm, called guided differential evolution. The guided differential evolution algorithm combines the differential evolution optimization technique with a cavity prediction algorithm . The use of predicted cavities during the search process, allows for a fast and accurate identification of potential binding modes.**[12]**

The advanced computing method solves the docking process less than 2 minutes in case of a single ligand and an enzyme. This fits also depend upon the molecular weight and presence of torsions in the ligand. The docking methodology as follows:

The enzyme and the ligands were imported into the virtual screen of the computer display. Prior, it should be checked whether enzyme is refined or not. Cofactors can be kept as such with the molecule space since it has simulated as like physiologic system.

The step involved in *insilico* docking studies is as follows:

Preparation: Before preparing the enzyme molecule for docking, the space searching for ligand on the active site has to be setup. After setting up the search space setup then the enzyme and the ligand(s) can be prepared ,followed by cavities has to be detected where active site, that is the groove for entry of ligand into the space which has been set up prior to preparation.

Docking: Docking wizard is a step by step process which involves choosing of ligands among the set of ligands, if multiple ligands were used. Next scoring function and binding site has to be defined ,in this investigation Mole Dock Score[GRID] was selected a grid resolution of 0.30(Å) ,which is an optimum value for scoring function. According to the search defined and grid set up for the binding site, the ligand can be evaluated for its internal electrostatic force, internal Hbond and Sp^2-Sp^2 Torsions. Followed by search algorithm to be customized, MVD uses four types of search algorithm viz., Mol Dock optimizer, Mol Dock SE, Iterated simplex and GPU screening using CUDA. Mol Dock SE as customized search algorithm was selected and carried out the docking process. During this customization constrains to different poses of the ligand inside the cavity are assigned then the docking process is optimized for H-Bonds and energy minimization. Various parameter settings with 1500 maximum iterations, 50 pose generation, energy threshold of 100 with maximum and minimum tries and simplex evolution of 300 maximum steps with a neighbour distance factor of 1 has been customized. Pose clustering on return multiple poses for each

run has been selected with enabling -5.00 energy threshold, clustering similar poses of RMSD threshold (1.00) with ignoring similar poses for multiple runs. Finally errors and warnings dialogue box shows no errors or warning, then setting up execution of docking to run docking in separate process and data output is retrieved from the storage MVD data files.

RESULTS

Maximum of 5 poses with best rank scores of every individual ligand on docking at active site of beta secretase is listed out in table1. Among the ligands used, gallocatechin-3-gallate shows lowest binding energy with MolDock score of -155.382kj/mol. Followed by (+)-Catechin-3-gallate (-142.625 kj/mol), Hesperidin(-132.942 kj/mol),Chrysin(-104.914 kj/mol) Delphinidin (-120.838 kj/mol) Epicatechin-3-Gallate (-131.838 kj/mol), Genistein(-114.854 kj/mol) Hesperitin(-113.185 kj/mol) . Beta secretase inhibitor-IV used as standard showed comparatively low binding affinity (-39.245 kj/mol). Fig 2 ,3 and 4 shows the interaction of amino acids at the active site with that of ligands during the docking process. on doing overlapping analysis, moreover it has been found that all ligands oocupied the hydrophobic site (fig 5) with high affinity. Fig 6 shows the binding interactions of reference standard at the active site of beta secretase. Binding site analysis is dealt in discussion.

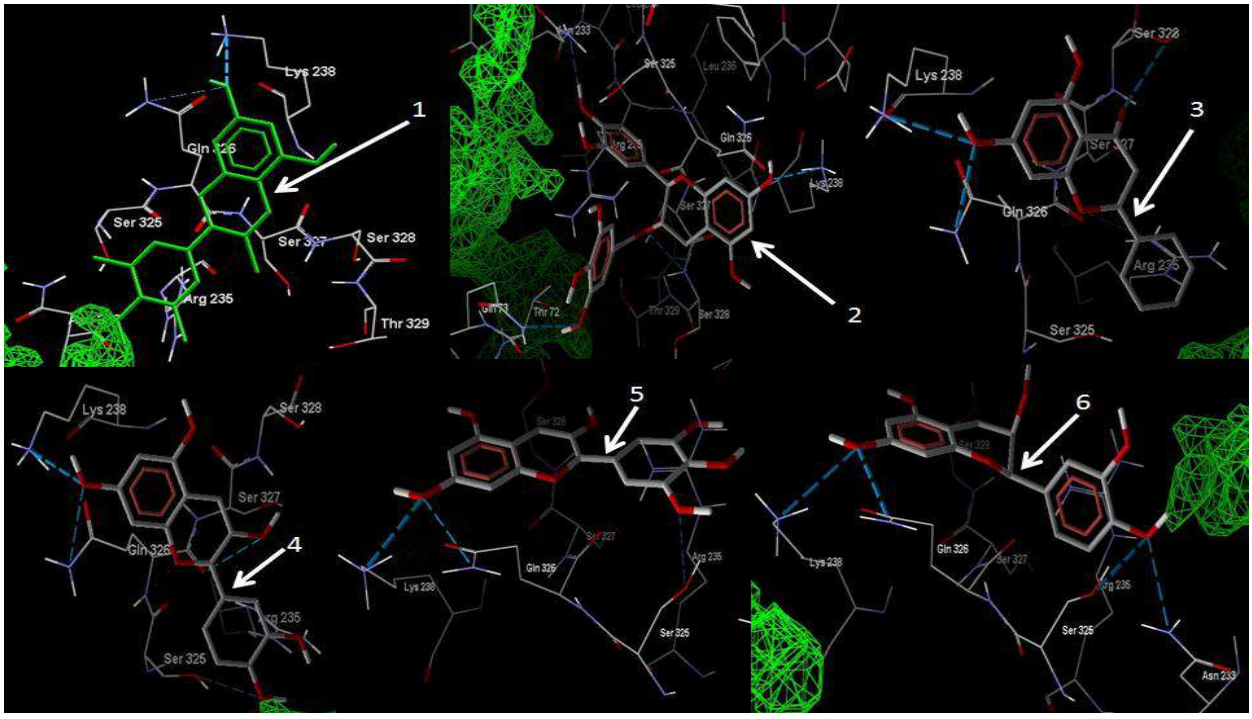

Fig 2. The binding sites of docked Polyphenolic compounds *(1.Anthocyanidin, 2.catechin-3-gallate, 3.chrysin, 4.cyanidin, 5.delphinidin, 6.epicatechin)* on the active site of BACE1 using MVD 6.0

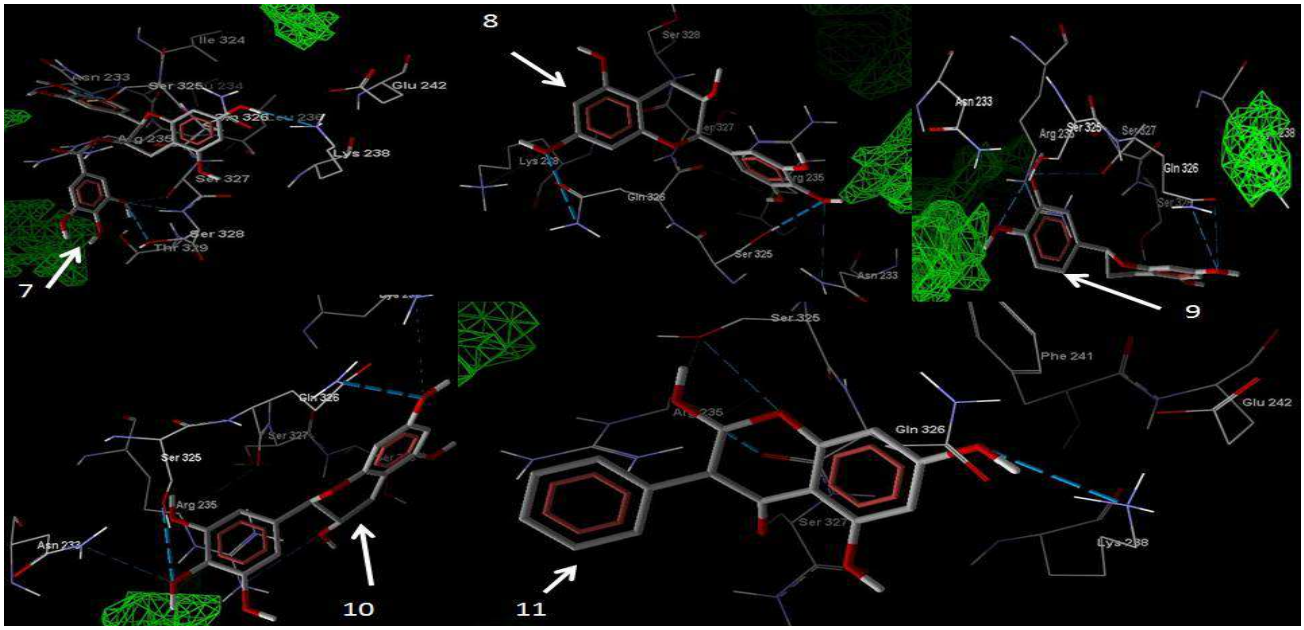

Fig 3.The binding sites of docked Polyphenolic compounds *(7. (-)-Epigallocatechin-3-Gallate, 8.Eriodictyl, 9. (+)-Gallocatechin, 10. (+)-11.Gallocatechin-3-Gallate)* on the active site of BACE1 using MVD 6.0

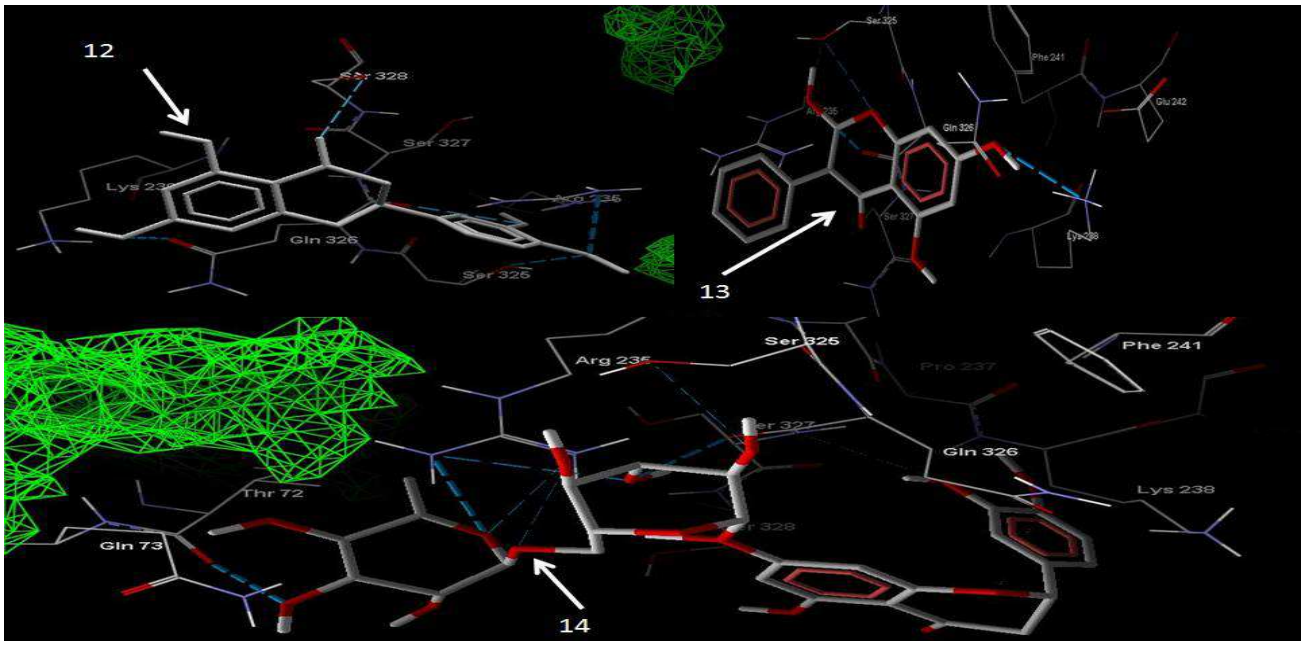

Fig 4. The binding sites of docked Polyphenolic compounds *(12.Genistein, 13.Hesperidin,* Hesperitin*)* on the active site of BACE1 using MVD 6.0

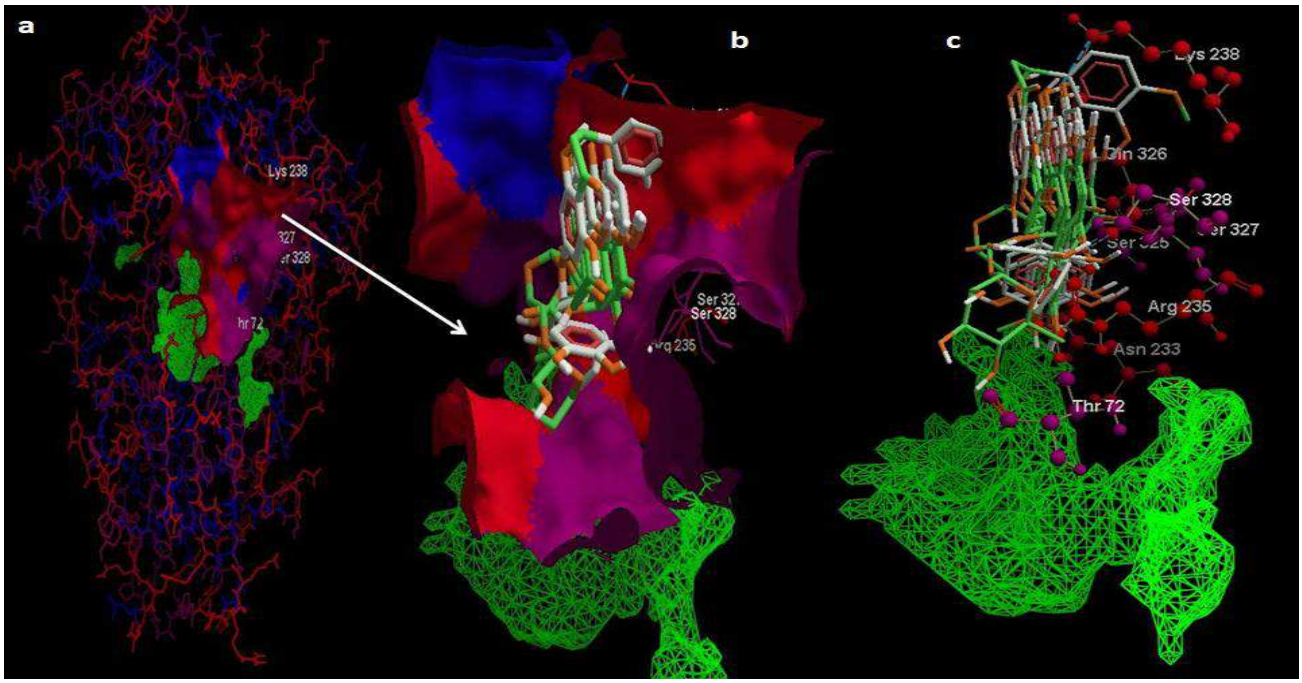

Fig 5. Crystal structure of BACE 1 showing its a) active site cavity with hydrophobic and hydrophilic areas b) Active site showing the hydrophobic areas with their amino acids along with the ligand set mentioned in table 1& 2. c) Amino acid involved in the interaction with the ligands is shown as a whole.

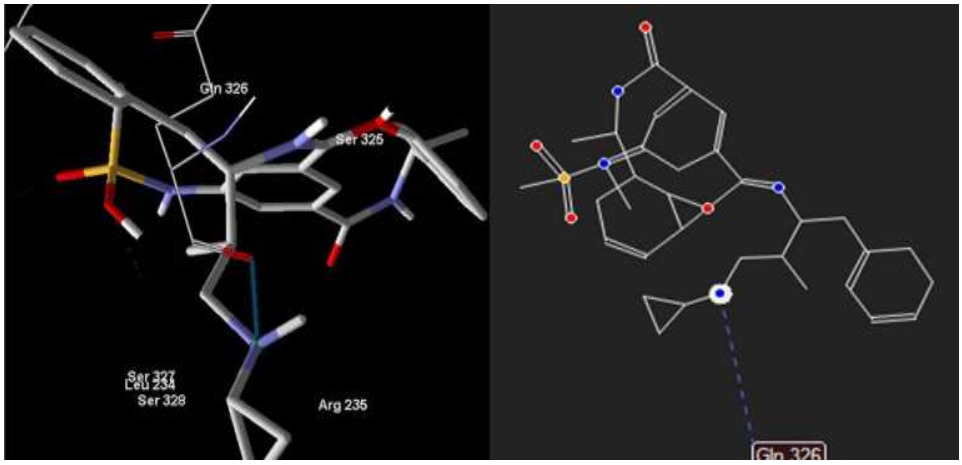

Fig 6. Figure showing the interaction of beta secretase inhibitor IV with Gln236 of beta-secretase

Table1. A list of binding energy (Mol Dock Score) of the selected ligands against BACE1 using MoleGrow Virtual Docker 6.0

S.No	Ligand	Molecular Formula	Molar Mass g/mol	Mol Dock Score Kj/mole
1.	Anthocyanidin	$C_{15}H_{11}O$	207.247	-79.4711
2.	(+)-Catechin-3-gallate	$C_{22}H_{18}O_{10}$	442.37	-142.625
3.	Chrysin	$C_{15}H_{10}O_4$	254.24	-104.914
4.	Cyanidin	$C_{15}H_{11}O_6$	287.24	-88.7246
5.	Delphinidin	$C_{15}H_{11}O7$	303.24	-120.838
6.	(-)-Epicatechin	$C_{15}H_{14}O_6$	290.27	-108.072
7.	(-)-Epicatechin-3-Gallate	$C_{22}H_{18}O_{10}$	442.37	-131.838
8.	(-)-Epigallocatechin-3-Gallate	$C_{22}H_{18}O_{11}$	458.37	-108.94
9.	Eriodictyl	$C_{15}H_{12}O_6$	288.25	-107.785
10.	(+)-Gallocatechin	$C_{15}H_{14}O_7$	306.27	-104.967
11.	(+)-Gallocatechin-3-Gallate	$C_{22}H_{18}O_{11}$	458.373	-155.382
12.	Genistein	$C_{15}H_{10}O_5$	270.24	-114.854
13.	Hesperitin	$C_{16}H_{14}O_6$	302.27	-113.185
14.	Hesperidin	$C_{28}H_{34}O_{15}$	610.56	-132.942
15.	Beta secretase inhibitor-IV*	$C_{30}H_{33}O_5N_4S_1$	578.7	-39.2455

**Beta secretase IV is an Isopthalide compound containing hydroxyethylamine (N-(1S,2R)-1-Benzyl-3(cyclopropylamino) -2-hydroxypropyl)-5-(methyl(methylsulfonyl)amino-N'-((1)-1-phenylethyl)isophthalamide) , CAS 797035-11-1, Calbiochem® Since a perfect beta secretase inhibitor has not been introduced in the market still under clinical trials. So a investigational beta secretase inhibitor Calbiochem has been used as a standard.*

Table 2. List of aminoacids,Hydrogen bond length between the atoms of ligand and amino acid with their interaction energy

S.No	Ligand	Amino acids interacted	Hydrogen bond length Å	Energy kj/mol	S.No	Ligand	Amino acids interacted	Hydrogen bond length Å	Energy kj/mol
01	Antho- Cyanidin	Lys 238 Gln 326	2.7 Å 3.3 Å	-2.5 -1.0	**09**	Epigallo- Catechin-3- gallate	Gln326 Ser325 Arg235 Lys238 Asn233	2.1 Å 2.9 Å 2.6 Å 3.1 Å 1.3 Å	-1.60 -0.69 -0.92 -1.95 -1.18
02	Catechin 3 gallate	Lys 238 Gln 326 Asn233 Gln73 Ser327 Ser328	2.5 Å 3.0 Å 3.1 Å 3.2 Å 3.1 Å 2.9 Å 2.9 Å	-1.7 -2.5 -2.0 -1.9 -2.2 -2.5 -1.1	**10**	Eriodictyl	Gln 326 Ser 328 Ser 325 Arg235	3.3 Å 2.6 Å 3.4 Å 3.3 Å 2.3 Å	-1.21 -0.49 -0.68 -1.14 -045
03	Chrysin	Lys 238 Gln 326 Ser 328 Arg235	3.0 Å 2.7 Å 2.8 Å 3.3 Å	-2.5 -2.5 -2.5 -0.4	**11**	Gallocatechin	Gln326 Ser325 Arg235	3.3 Å 2.9 Å 2.1 Å	-1.34 -0.69 -0.61
04	Cyanidin	Lys 238 Gln 326 Arg235	2.6 Å 3.3 Å 2.9 Å 3.4 Å	-2.5 -1.3 -1.3 -0.5	**12**	Gallocatechin 3gallate	Lys 238 Gln326 Ser325 Asn233 Gln73 Thr72 Ser328	2.5 Å 3.1 Å 3.2 Å 3.0 Å 3.1 Å 2.8 Å 3.4 Å 3.1 Å	-2.36 -1.14 -1.28 -1.08 -2.05 -0.63 -1.32 -1.26
05	Delphinidin	Lys 238 Gln 326 Arg235	2.7 Å 3.2 Å 3.2 Å	-2.5 -1.2 -0.6	**13**	Genistein	Lys238 Ser 325 Gln 326	3.2 Å 1.8 Å **2.0** Å 1.9 Å	-1.86 -1.24 -2.64
06	Epicatechin	Lys 238 Gln 326 Asn233 Ser 325	3.2 Å 2.8 Å 3.3 Å 2.6 Å	-1.92 -2.50 -1.49 -2.5	**14.**	Hesperitin	Gln 73 Thr 72 Gln326 Arg 235	1.1 Å 2.9 Å 2.5 Å 3.1 Å	-10.31 -2.56 -1.52 -1.85
07	Epicatechin 3gallate	Arg235 Asn233 Lys238 Ser 325 Ser 237 Ser 328 Gln 326	2.6 Å 1.2 Å 3.1 Å 2.5 Å 3.4 Å 2.4 Å 3.1 Å 2.3 Å	-0.81 -1.22 -2.03 0.94 -1.9 -1.16 -1.30 -0.41	**15.**	Hesperidin	Gln326 Ser 325 Ser 328 Arg235	2.9 Å **2.8** Å 3.1 Å 3.3 Å 3.1 Å 3.4 Å 3.5 Å 3.3 Å	-2.5 -2.5 -2.0 -1.33 -2.1 --0.4 - 0.3 -0.1
08.	Epigallo- Catechin3gallate	Gln326 Ser325 Arg235 Lys238 Asn233	2.1 Å 2.9 Å 2.6 Å 3.1 Å 1.3 Å	-1.60 -0.69 -0.92 -1.95 -1.18	**16.**	**Beta secretase inhibitor IV Calbiochem®*** (Standard)	Gln326	1.1 Å	-1.15

DISCUSSION

The binding energy determines the intensity of interaction between a ligand and an enzyme. The lowest binding energy is the outcome of the best conformer at its receptor site or active site of an enzyme. Most of the ligands used in this work have comparatively acceptable binding energies to the standard used. Almost all docked ligands posses significant lowest binding energy of Mol Dock Score within the the range of **-79.4711 –to 155.382 kJ/mole.** Those compounds with high molecular weight (with in these classes) and as well as considerably more hydroxyl groups involved in interaction with the amino acids with increased fidelity. The binding energy as well as the hydrophobic groups embedded in the cavity area makes the ligands to keep their foot on the hydrophobic area and 10% of their ligand interacts with the aminoacid at the hydrophilic surface.

The ligands have some specificity towards the aminoacids at the hydrophobic pockets. Next to the hydrophobic areas containing aminoacids and nearby adjoining aminoacids are responsible for the interaction of the ligands. These ligands search their spaces provided in and around the cavity. From the diagram it is clear that these ligands has their area of interaction with the hydrophobic areas with amino acids like **Thr72 ,Gln73, Asn233, Arg235,Lys 238, Ser 325, Gln 326, Ser327 ,Ser328 .**

A structurally similar interaction was found to be existed among the ligands. The amino acids like Gln326, Arg235, Lys238 has its interaction with most of the Polyphenolic compounds.

The crystal structure of beta secretase the crystal structure of the protease domain of human memapsin 2 complexed to an eight-residue inhibitor at 1.9 angstrom resolution.The active site of beta secretase is more open and less hydrophobic than that of other human aspartic proteases. The subsite locations from S_4 to S_2' considered to be important in developing a ligand that inhibit BACE1.Studies found out that hydrophobic pocket of the enzyme play an important role in substrate binding. Non-peptidic ligands targeting hydrophobic pocket residues should inhibit beta secretase cleavage.**[13] Catechin3gallate** at its lowest binding energy -142.625 kj/mol interact with Lys238, Gln326, Ser327, Ser328, Asn233, Gln73. oxygen atom of C7 substituted hydroxyl group interacts with the Lys 28 (2.7 **Å**) and Gln 326(-1.0 **Å**) at the same time the fused ring system containing oxygen next to C3 makes similar hydrogen bond (length) with Ser327 (2.9 **Å)** and Ser328 (2.9 **Å)** nearby residues. As the gallate group has a flexibility at the fused ring system, makes a turn and interact with Asn 233 (3.1**Å)**, whereas hydroxyl groups in the gallate has interaction with the hydrophilic residue area Gln 73(3.2**Å). Delphinidin** (-120.838 kj/mol) also has the same type of interaction but devoid of interaction with Asn233 because at C3 there is a lack of gallate moiety which is essential in this positional arrangement of ligands. **Chrysin** makes its binding energy -104.914 kj/mol at the active site and fit along with a conformation in that position their C7 attached hydroxyl group containing oxygen atom interact with lys238(3.0 **Å**) and Gln326(2.7 **Å**). The oxygen atom of ketonic group (C=O) at the C4 position to form a Hbond with Ser328 (2.8 **Å**) at right angle to the above mentioned aminoacid residues. The oxygen atom between C2 and C8 interacts with nitrogen atom of Arg 235 (3.3 **Å**) to produce electrostatic interaction as **Chrysin-O…NH2**-(HN=C)-NH-ARG.**Cyanidin,** hydroxyl (-OH) groups C3 and C7, C4′ has Hbond interaction with Gln326 (2.6 **Å**), Lys238 (2.9 **Å**), Ser 325 (3.4 **Å**) at free energy of binding (-88.7246 kj/mol). The oxygen atom in fused cyclic system next to C2 of the Cyanidin has its Hbond interaction with terminal peptidyl linkage of Gln326(3.3 **Å)**.**Epicatechin-3-Gallate,** the levorotatory form of epicatachin differs from catechin 3 gallate in its interaction with Ser325(2.5 **Å**) at final intermolecular binding energy -131.838 kj/mol. So that there is a continuous interaction of amino acids Ser235,Ser 237,Ser 238,where Lys 236 goes inward conformation without any binding with the ligand.**EpigalloCatechin-3-gallate** with -108.94 kj/mol as binding energy produces an interaction like of mixed catechin 3 gallate and epicatachin .The levorotatory form of this ligand has its maximum hydrogen bond interaction with amino acid, residues Gln326 (2.1 **Å**), Ser325 (2.9 **Å**), Arg235 (2.6 **Å**), Lys238(3.1 **Å**), Asn233(1.3 **Å**).**Eriodictyl** has the interaction character as like that of hesperitin with the contribution of continuous amino acids Ser 325(3.3 **Å**), Gln326(3.3 **Å**; 2.6 **Å)** , Ser328(3.4 **Å**) at its lowest binding energy -107.785 kj/mol. But the oxygen and hydrogen atom of C7 bearing hydroxyl group share its Hbond with terminal peptide H_2N–C=O-Gln326.**Genistein** with a satisfactory binding energy **(-114.854 kj/mol)**. Ser 325(1.8 **Å**) terminal CH_2OH Hbond with oxygen atom of O1 and C2 hydroxyl group, Gln326 (1.9 **Å**), Lys 238(3.2 **Å**), again the involvement of continuous aminoacid sequence explain the general mode of interaction.**Gallocatechin** when compared with other ligands in this investigation gallocatechin contains C4' hydroxyl group which has an exceptional Hbond with Asn233 at its binding energy (-104.967kj/mol). Simultaneously the same hydroxyl group containing oxygen share with Ser325 and the ligand extends its axis towards the same line and interacts with the Gln326 through C7' hydroxyl group. Positioned structure in this conformation, C7′ hydroxyl group Hbond with terminal NH_3 group of

Lys238.**Gallocatechin-3-gallate** containing trihydroxy nature of gallate moiety attached at C3 position has its interaction Thr72,Gln73. The Oxygen atom linking between gallate and fused ring system, Hbond with hydrogen of terminal hydroxyl groups ser327 and NH_2 group of Ser328. Whereas the C2 trihydroxy gallate group Hbond with C3′ ser325 and at the same time**.Hesperitin** interaction at BACE1 active site with free energy of binding -113.185 kj/mol but with more than three Hbond formation due to the structure of hesperitin hydroxyl groups and as well as their positioning at the hydrophobic site. In such a conformation a continuous amino acids(excluding the intermediate ser327 because the intervening –NH group peptide bond changed its conformation) on lipophilic area viz.,Ser325(3.1 **Å**),Gln326(2.9 **Å**),Ser 328 (3.3 **Å**) has interaction through Hbond with oxygen atom of C4' and C5' hydroxyl groups whereas (ketonic C=O) of C4 of the fused ring sytem respectively. On the other side, oxygen atom of hydroxy group at C4' as well as share its interaction through Hbond with the terminal guanidine moiety of Arg 235(3.0 **Å**). At the other side hydroxyl group attached to C7 of hesperitin , Hbond with Gln326(2.8 **Å**) with its terminal (-C=O) group , further the ligand stretches the hydrophobic surface more inward . **Hesperidin is** (2S)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxy-2,3 dihydro chromen-4-one, a glycone of hesperitin containing two glycones interlinked by a methoxy (- CH_3) bridge. It makes a delicate interaction with residues at the hydrophobic site due to its elongated structure and flexibility of rings forward ,backward and bent along their plane in order to go with the affinity of the functional groups attached making its free energy of binding (-132.942 kj/mol) and hence more stronger. The guanidyl group of Arg 235(3.1 **Å;** 3.4 **Å**;3.5 **Å** ;) makes four Hbond with the terminal glycone moiety containing oxygen atom next to the interlinking methoxy group of preceding intermediary glycone moiety. Among the four Hbond (3.3 **Å)**, one of the hydrogen is made with the oxygen atom of methoxy group. The terminal glycone group containing hydroxyl groups has its interaction with the Ser325 (3.1 **Å**), Gln326 (**2.8 Å**), Ser328 (3.3 **Å**).

The aminoacid residues considered to be responsible for major interaction with the ligands were Thr72,Gln73,Asn233,Arg235,Lys238,Ser325,Gln326,Ser327,Ser328. These ligands occupy the major sites where the aspartic acid residues of amyloid precursor proteins has its interaction with BACE1.

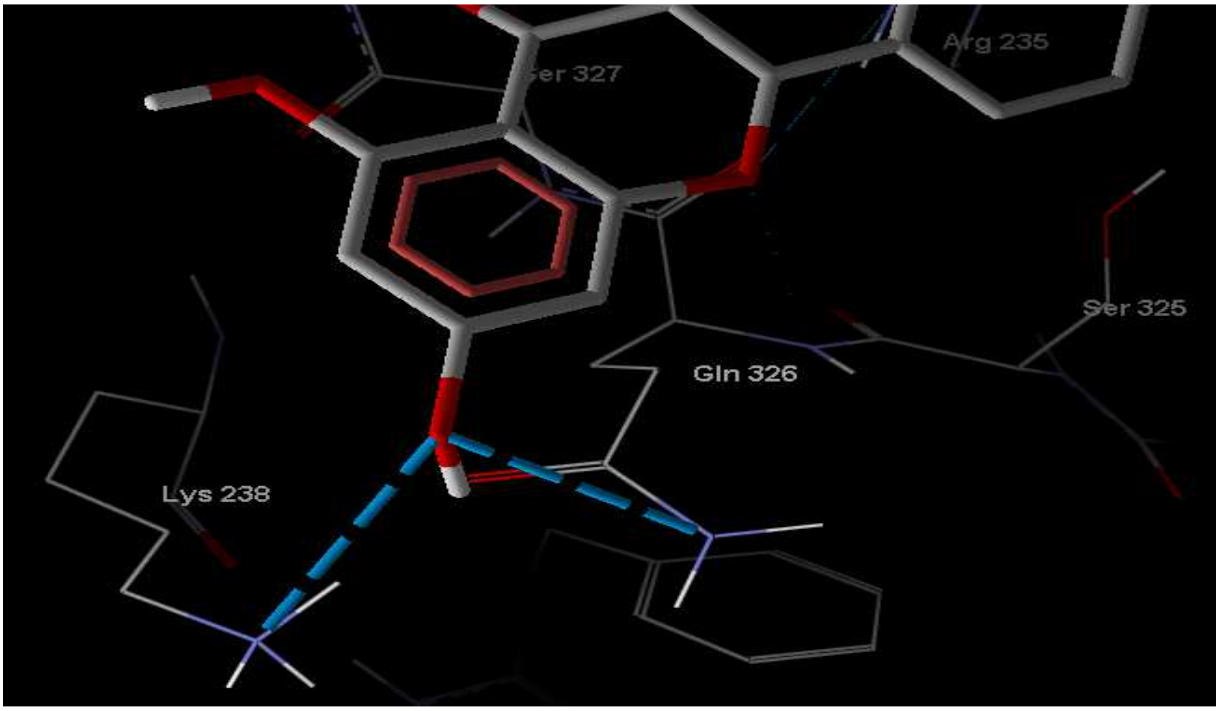

Fig 7: Shows the way of catechin 3 gallate positioning (most of the ligands of these classes)on the hydrophobic area containing Lys238 and Gln326 to make Hbond with Oxygen atom of hydroxyl group at C7 at their lowest binding energy.

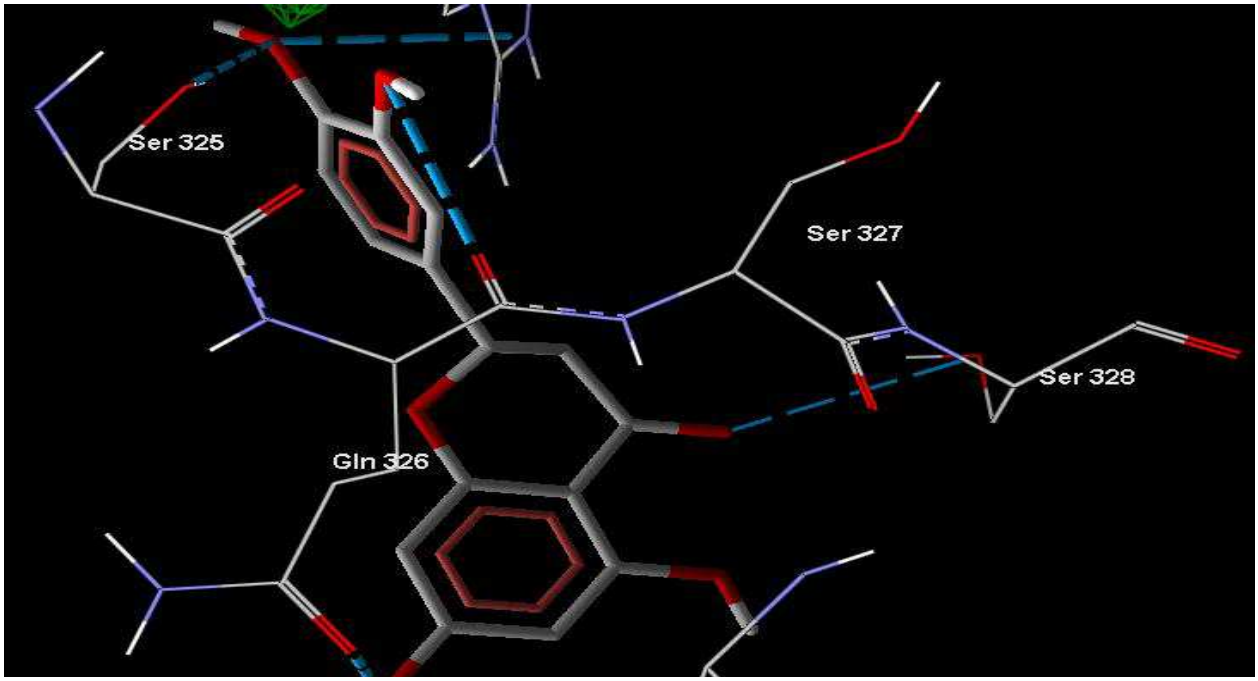

Fig8: Shows the interaction of continuous amino acid structure through Hbond formation with hydroxyl groups at C4', C5',C4 of hesperitin at the active site cavity.

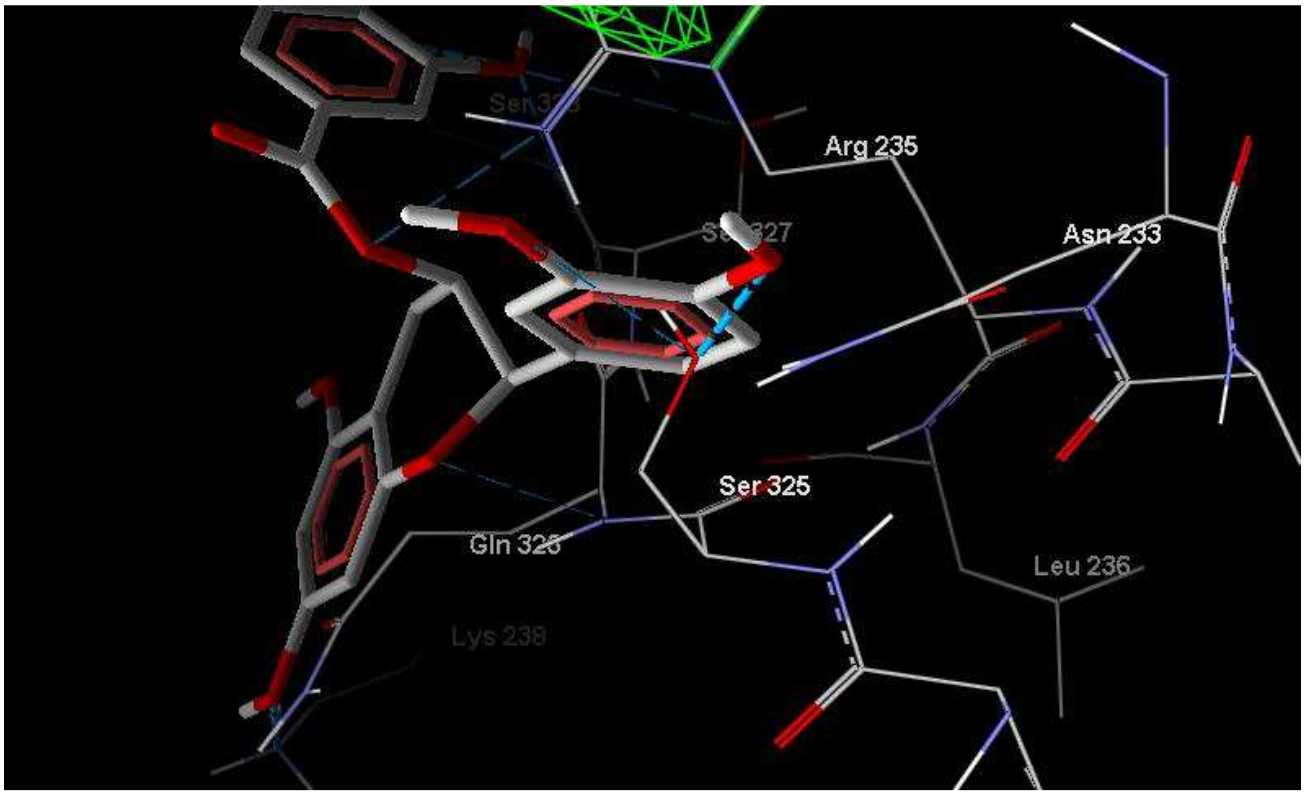

Fig 9: Shows the interaction of Ser 235 with levorotatory form of epicatachin 3 gallate which was absent in Catechin-3- gallate.

CONCLUSION

Binding site analysis has revealed the effective affinity of the polyphenolic compounds at the active site. Lead molecules can be developed from the polyphenolics on account of their mode of binding with the active site residues. From the lowest binding energies and hydrogen bond formation, its length with the amino acid residue makes Polyphenolic compounds to show a high degree of interaction with the active site of BACE1. These compounds can be undergone for further Invitro enzyme inhibition and *in-vivo* studies for their activity against beta secretase will be helpful for finding out a drug of better therapeutic drug against Alzheimer's disease.

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